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CRYPTOCOCCUS PNEUMONIA IN EQUIDÆ.

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I. INTRODUCTION.

THE object of this paper is to give a summary of observations recently made in the Sudan on a hitherto unrecorded type of infection with *Cryptoccoccus farciminosus*, namely, an apparently primary pneumonia.

It must be admitted that this definition of the phenomenon may not be entirely accurate. In the first place the organism at issue has not been in every possible way identified as *C. farciminosus*, but is provisionally taken to be such on account of its identical morphology and staining reactions and the character of the histological changes in infected lung tissue. Secondly, it is possible that in all such cases the lung is not the primary seat of infection, but reference to the appended notes on individual cases must lead to the conclusion that in most instances it is ; in any case the lung infection is in quite a different class from those that have on rare occasions been recorded as resulting from long-standing generalised epizootic lymphangitis. Finally, the lung lesions may possibly not be regarded as typical of any recognised form of pneumonia, since the changes are essentially of an infiltratory nature and the usual phenomena characteristic of inflammation are not represented ; in particular, the process is remarkable for the strong inhibition of attempts at repair. It is, however, convenient to consider the process broadly as a primary interstitial pneumonia, and to assume, until evidence to the contrary is forthcoming, that the causal organism is *Cryptococcus farciminosus*.

It is considered that the phenomenon has hitherto escaped notice

largely because, in the absence of superficial lesions, it would be unlikely that any association would be conceived between obscure respiratory trouble and epizootic lymphangitis; or alternatively, assuming that lung infection may be associated with superficial lesions, it would be unlikely that on destroying an animal suffering from epizootic lymphangitis a post mortem examination including inspection of the lungs would be carried out. Further, as will be appreciated on reference to the observed post mortem and histological changes, it seems highly probable that the phenomenon has been seen, but in circumstances that would make etiological diagnosis possible.

It cannot be claimed that the number of cases observed in the dan is sufficient for a final account of the condition to be given, but for the purpose of this communication it is convenient to give a tentative general description, supporting this with an appendix comprising notes on individual cases.

II. Symptoms.

All cases hitherto recorded have been in horses and mules, no case in a donkey having yet been seen. In consideration of the equal susceptibility of donkeys to cryptococcus infection, there are probably no reasons for this hiatus other than the small total number of observed cases and the fact that donkeys do not receive much official attention.

In general the symptoms noted have merely been of indefinite pulmonary trouble, in which a tentative diagnosis has been made; in all cases but one the respiratory symptoms have been, as far as can be ascertained, unassociated with either co-existent or antecedent superficial lesions of epizootic lymphangitis.

The symptoms have varied slightly in individual cases, but the main feature, either on first examination or later in the disease, has been accelerated and increasingly shallow respiration, tending in the later stages to become markedly abdominal. In one case only has a history of coughing been recorded.

In the cases in which temperatures were taken there was a history of mild intermittent fever, the usual feature being a series of febrile periods each of a few days' duration, recurring at irregular intervals, and not characterised by any great elevation of temperature.

There was considerable loss of condition in the cases which were allowed to become advanced, although with the exception of a slackening during the febrile periods the appetite remained good throughout.

It is not possible to estimate with any degree of confidence the length of time over which these symptoms may continue, and reference to the morbid anatomy will show that it will certainly vary within wide limits. Taking the extreme cases in the present series, one resulted in death about three weeks after the date of the first observation of respiratory symptoms, while at the other end of the scale is a mule that was kept under observation for four months before it was destroyed, and even after this period it did not appear to be imminently threatened with death.

In consideration of these symptoms it is therefore not irrelevant to consider whether, in a country in which trypanosome infections are common, some few cases of cryptococcus pneumonia have not been suspected of and even destroyed for trypanosomiasis.

III. Post Mortem Appearances.

Two striking features become evident after the examination of a relatively small number of cases ; first, the great variety encountered both in extent and distribution of the lesions in the lungs, and second, the marked tendency of the disease process to remain confined to the lung, without extending to the pleural cavity or the bronchial glands even in the most advanced stages. Further observations may show the latter not to be constant, but in regard to variety of distribution and degree of infection the condition is comparable with bovine or human pulmonary tuberculosis.

One lung or both may be affected, and the infected area may consist of a single circumscribed lesion or be distributed evenly or unevenly throughout the pulmonary tissue.

In either case it is first noted that there is no visible congestion, either general or in the neighbourhood of infected areas, and that in proportion to the degree of infection and the area over which it extends the lungs are abnormally pale. In cases where the condition

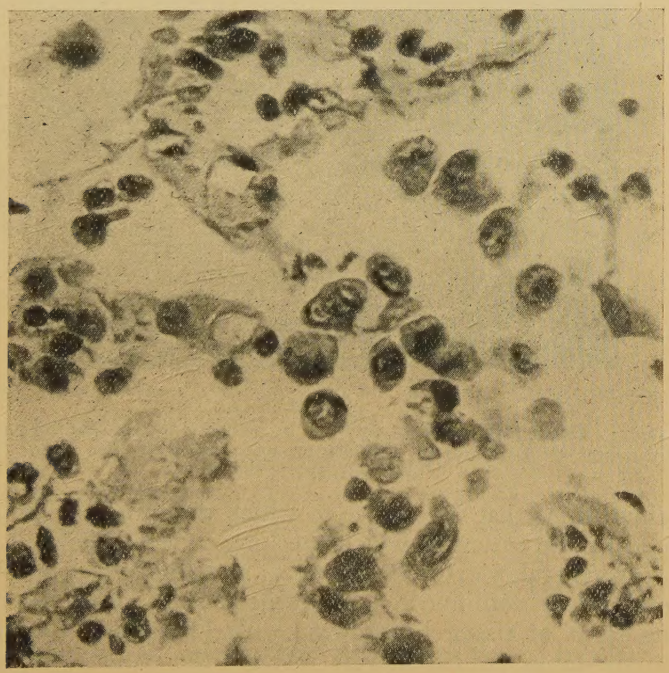

Fig. 1.

Early stage showing the monocytic appearance of most of the cells, although some lymphocytes remain. Hæmatein and eosin. × 70.

is both extensive and advanced large areas of lung may be almost dead white in colour, giving on incision an impression both in appearance and consistency of a mass of soft white indiarubber. In less advanced cases the pale areas are of irregular sizes and shapes, ranging from a barely appreciable spot, usually associated with a small bronchus or interlobular septum, to the conditions already described.

On squeezing the cut sections of lung it is in most cases difficult to express any fluid, although by making several incisions areas may be found that will yield a small quantity of creamy material. When obtained, this material will be found on microscopic examination to contain a greater or smaller number of cryptococci, but smears from infected tissue in the absence of such exudate may often be examined with great thoroughness without a single organism being detected. It has been noticed that the more extensively and uniformly a lung or area of lung is invaded, the less likely it is for organisms to be detected in tissue smears ; in lungs, however, in which the process is imcompletely or irregularly distributed some areas may reveal the presence of cryptococci in immense numbers.

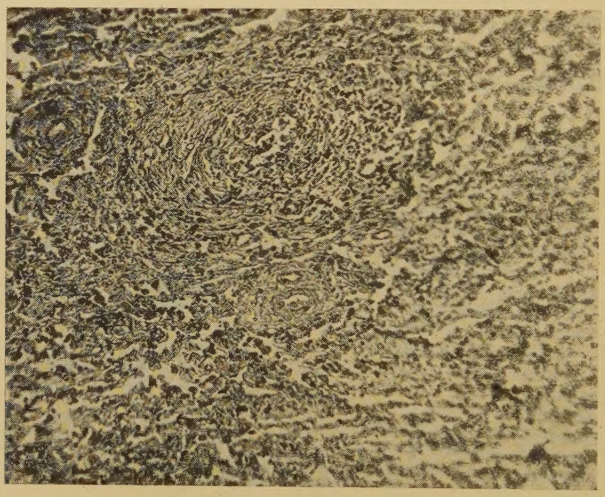

FIG. 2.
Second stage. Dense infiltration involving a bronchus and surrounding parenchyma. A parabronchial artery showing obliterating endarteritis is also seen. Hæmatein and eosin. × 50.

It thus seems that the degree to which the infection can progress is limited by the extent to which the lungs are invaded ; if invasion is not very general or extensive there is a possibility that in some areas the process may continue to an advanced stage, included in which is some form of tissue breakdown with liberation of cryptococci, while if the lungs are extensively invaded the patient may succumb before this stage is reached in any area.

The pleura, in cases where the infection involves it, is thickened, but the deep layers alone seem to be affected, since in all cases

hitherto examined the surface has been glistening and adhesions absent. In advanced cases thickened processes are seen extending inwards apparently along the lines of interlobular septa ; they can, however, be followed for a short distance only, owing to the similar appearance of the tissue into which they extend.

The bronchial and mediastinal glands, in cases where a note has been made of their appearance, have been normal or at most slightly enlarged. It may be presumed that in the remaining cases a similar condition obtained, since if they had been abnormal the fact would have been recorded.

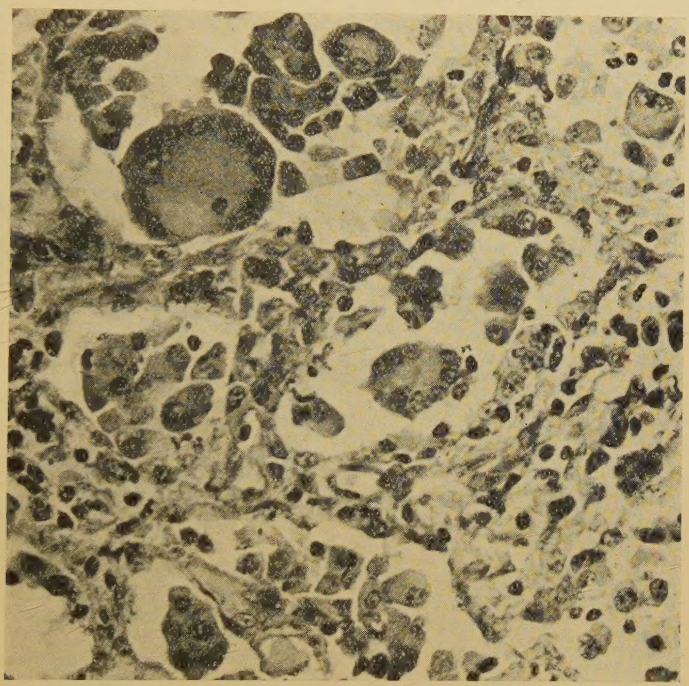

FIG. 3.
Third stage. Syncytia and giant cell formation before the appearance of parasites. Hæmatein and eosin. × 450.

Finally, it is worthy of note that in no case in this series has the post mortem examination revealed any associated abnormality in any of the other viscera ; in only one instance was there a history of superficial epizootic lymphangitis, and in this case no connection between the two could be found on post-mortem examination.

IV. HISTOLOGY.

In spite of the great variability in the naked eye appearance of the lesions, little difficulty has been found in working out the course of histological changes, or at any rate the sequence of main changes. It

has sometimes been possible to follow every stage of development in a single specimen, a procedure that is not difficult in a lung showing an irregular distribution of lesions, but one that entails the microscopic examination of a number of preparations from different areas. In other individual cases, particularly those in which the disease process was fairly uniformly distributed, no amount of selection of tissue has made it possible to follow the complete series of changes, but the identity of such changes as were seen with those occurring in the more complete cases has served to confirm the whole.

At the present stage of the study it seems that the most convenient procedure in describing the changes is to divide them into four main stages. It is not to be understood, however, that these stages are clear-cut; they are at least overlapping, both in the sense that in any one field it is often impossible to define the condition as being

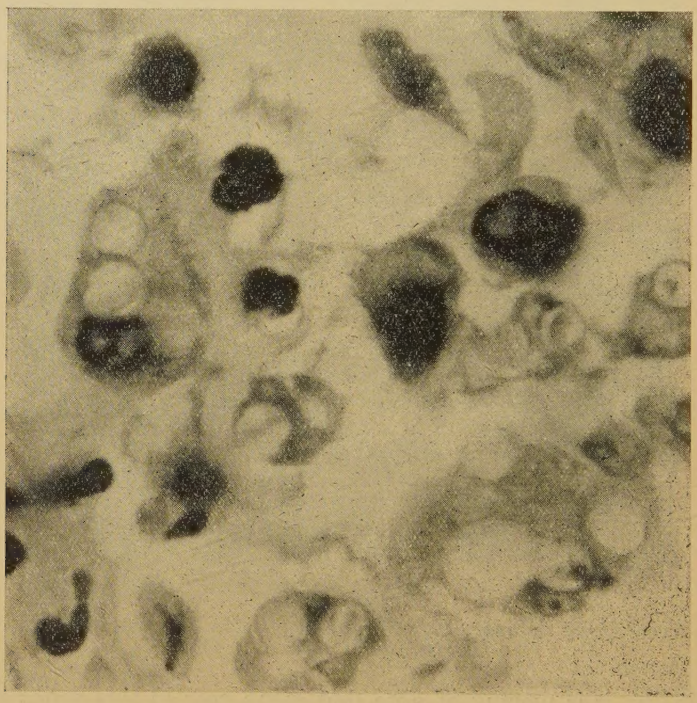

FIG. 4.
Late third stage. Appearance of cryptococci in the monocytes. Hæmatein and eosin. × 2,000.

entirely at a particular stage, and in the sense that in adjacent fields, usually in adjacent lobules, two widely separate stages are often seen side by side. There may be a number of changes, particularly in lungs in a fairly advanced stage of infection, that are obviously of a secondary nature; it is proposed to leave a consideration of these until the four main stages have been outlined.

First stage.—(Fig. 1). This stage can best be seen in pieces of lung selected from areas in which the smallest irregular white spots are seen on post-mortem examination, and is essentially an infiltration with small mononuclear cells. It is difficult to be certain of the actual site in which this infiltration is to be first observed in very early cases, but it seems that the terminal bronchi are the earliest structures involved. The infiltration commences in the peribronchial connective tissue, and, while increasing in this position, it extends into the interalveolar septa. This order of events is deduced from the fact that when an early lesion is studied the amount of infiltration around the small bronchus itself is invariably greater than that radiating from it.

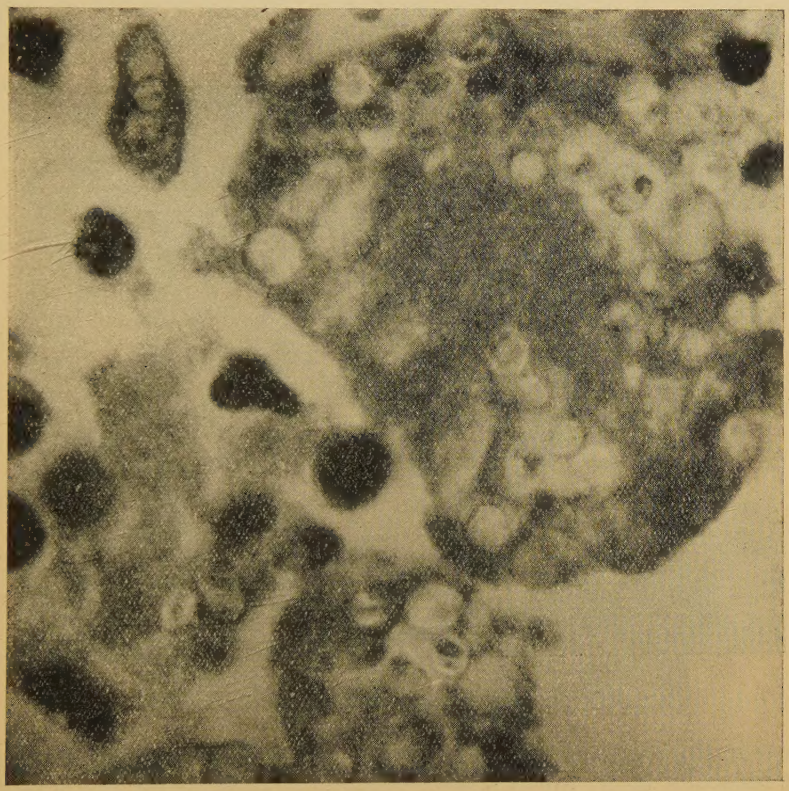

FIG. 5.
Fourth stage. Greatly swollen cells containing numbers of cryptococci. Hæmatein and eosin. × 2,000.

It is particularly noticeable at this stage that the bronchus and associated alveolar walls show no signs of congestion ; it is difficult to detect red blood corpuscles in the interalveolar septa, and neither the lumen of the bronchus nor the alveoli contain any exudate. This process appears to continue until a whole lobule or several lobules

become heavily infiltrated, although the general structure of the lung remains quite recognisable.

The infiltrating cells themselves seem to be entirely lymphocytes.

Second stage.—(Fig. 2). The essence of this stage is the replacement, or possibly the outnumbering, of the lymphocytes by large mononuclear cells. This process is necessarily accompanied by a still more dense infiltration of the originally invaded structures, in the course of which the walls of the small bronchi and associated vessels become greatly thickened and the alveolar spaces become smaller. This stage may advance to the obliteration of large numbers of small bronchi and alveoli, giving to considerable areas of lung an almost sarcomatous appearance. Generally speaking, however, it is not difficult in most areas in this stage to identify the material as lung tissue. It is not without interest to examine a series of small bronchi becoming progressively obliterated. The infiltration which

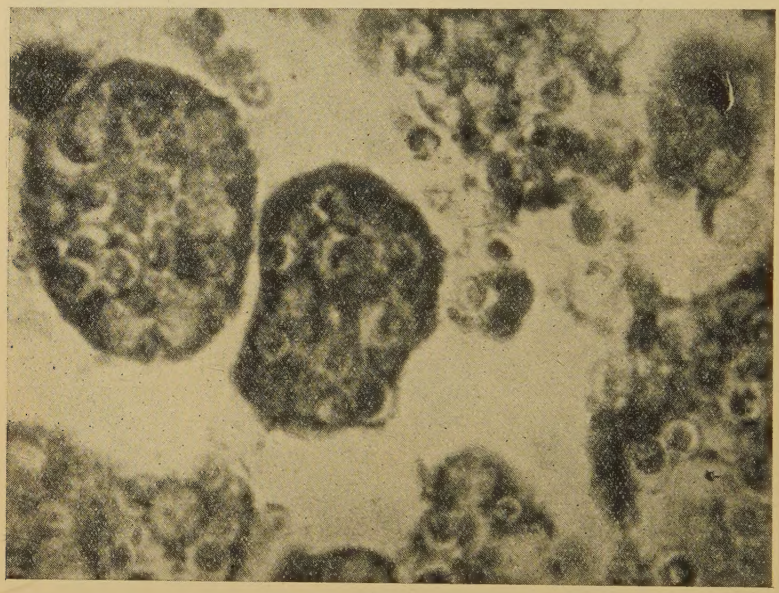

FIG. 6.
Same as Fig. 5. × 1,800.

starts in the region of the outer fibrous coat extends inwards through the muscular layer, which it soon obliterates, towards the epithelium. As the lumen becomes smaller the epithelial cells degenerate, the nuclei becoming indistinct and the individuality of the cells lost. Ultimately the epithelium completely disappears and the bronchial lumen is indistinguishable from the adjacent contracting spaces representing the alveoli. The parabronchial veins appear to undergo a comparable process, while the arteries, although ultimately becoming completely infiltrated and lost, show an intermediate stage of obliterating endarteritis. But at this point the third stage is becoming evident.

Apart from the actual infiltration it is again noticeable that, as in the first stage, there is no congestion or bronchitis and, with the exception of the final destruction of bronchial and alveolar epithelium, little catarrhal exudate.

In regard to the infiltrating cells that are characteristic of the second stage, these have merely been mentioned as " large mononuclear cells " to distinguish them from the lymphocytes that characterise the first stage. Certainly, with usual histological staining, they are indistinguishable from ordinary monocytes, and for the sake of convenience will henceforward be described as such. Considering, however, the position in which they occur and the tissues with which they are associated, it seems that they probably originate as wandering cells or are derived from the alveolar epithelium ; their lymphatic, connective, or endothelial origin can largely be negatived by an argument of exclusion. Although their origin as wandering cells seems most probable, it is worthy of note that in certain areas, in

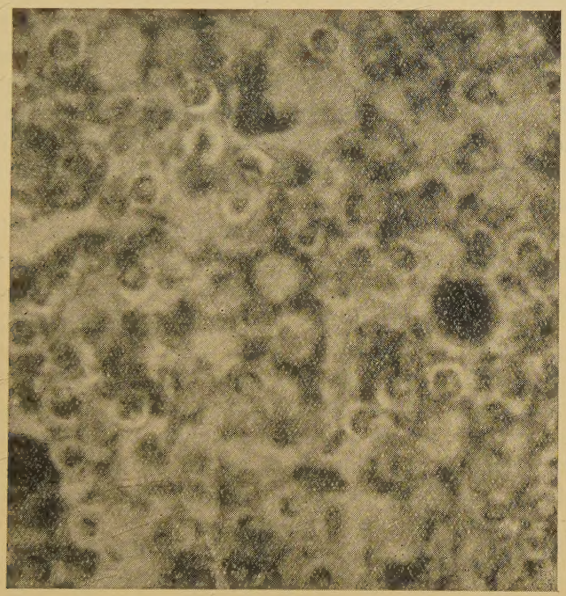

FIG. 7.

Late fourth stage. Complete replacement of cellular tissue by cryptococci. Hæmatein and alcoholic alum eosin. × 1,800.

which the infiltration has not yet become too dense, their arrangement in relation to what remains of alveolar walls suggests that some of them at any rate may be derived from the alveolar epithelium. These cells, however, do not comprise the whole of the cell mass. The lymphocytes that characterise the first stage remain in varying numbers, being especially persistent in the neighbourhood of the bronchi rather than in the inter-alveolar tissue, and in fact it is difficult to know whether they are anywhere replaced by the monocytes

or merely swamped by them in point of sheer numbers. In addition, a small number of plasma cells and polynuclears are present. The former, as to a certain degree in the first stage, are in relatively small numbers, and even staining by Pappenheim's pyronin-methylgreen method fails to make them stand out appreciably from the surrounding cells. The polynuclears are in even smaller numbers; it seems probable that they are mainly concerned in the removal of the debris representing the original lung tissue, as they are most numerous in the traces of exudate that are to be seen in some of the small bronchi.

Third stage.—(Figs. 3 and 4). The characteristic phenomenon of this stage may appear before the second stage is complete, namely, while a certain amount of lung structure is still recognisable.

The two main features of this stage are modification of some of the new monocytes and the appearance of cryptococci.

The most obvious modification of the monocytes is the appearance of syncytia with its usual series of changes. In certain densely infiltrated areas foci appear at which the cells lose their clear-cut outline and ultimately fuse to form multinuclear giant cells. Simultaneously in other monocytes cryptococci become visible. By ordinary histological methods of staining—*e.g.*, hæmatein and eosin—these appear as oval highly refractile bodies in the cytoplasm, and, while at first only one or two are to be seen in any individual cell, they later multiply until fifty, sixty, or more* are present before the cell finally disintegrates.

It seems that the "third stage" constitutes the crisis of the infection, being that at which the cryptococci begin to obtain the mastery and the invaded tissue turns from attack to defence. In the earlier stages the mononuclears, by combined phagocytosis and proliferation, appear to hold their own; but at the end of the second stage so dense a tissue has been formed that unrestrained multiplication is impeded and typical cryptococci appear. The formation of giant cells seems to be a movement of defence, but from the fact that they ultimately become invaded, and, as will be seen in dealing with the fourth stage, finally destroyed, it seems that defence is attended by no better results than the original attack.

In the third stage again, and apart from the changes just described, it is noteworthy that the usual phenomena of attempts at repair are negligible. Fibroblasts are extremely rare and by van Gieson's stain no new fibrous tissue can be seen. Rare new blood vessels may, however, be seen in certain areas.

Fourth stage.—(Figs. 5, 6 and 7). This stage presents the final mastery of the cryptococci. The organisms continue to invade the monocytes, and to multiply inside them, and, commencing somewhat later, the same process occurs in the giant cells. The invaded cells increase in size, in some cases up to ten or more times their original diameter, the nuclei ultimately cease to retain nuclear stains, and

* This refers to the number of organisms seen in a section of about three microns' thickness. A complete cell might contain some hundreds of cryptococci.

the outer limits of the cytoplasm become indistinct, so that a parasitised cell assumes the form of a mulberry like group of cryptoccocci. Finally the organisms become dissociated from these groups and lie indiscriminately in immense numbers in a structureless matrix.

Although at this stage the organisms can be readily recognised in preparations stained by ordinary histological methods, far better results can be obtained by staining the organisms themselves and leaving the tissue to a counterstain. Of the staining methods tried in this series of specimens that of Claudius has been found the best for demonstrating the cryptococci themselves although the Drew-Murray method gives almost as good if somewhat more delicate pictures. In fact, the cryptococci are so numerous and the cells are either degenerated or destroyed to such a degree that the most efficient stains are those that would be employed in preparing a smear of epizootic lymphangitis pus.

Consideration of the third and fourth stages is instructive in explaining the difficulty encountered in detecting cryptococci in smears made from fresh tissue. It seems that the lung infiltration constitutes a new, fairly strong cellular tissue, from which elements are not easily detached in the process of making smears. Further, even if a few cells are detached a lengthy search will be necessary for the detection of organisms if the infection is at any stage earlier than the one just described.

Changes in the Pleura.—The foregoing description is limited to the changes in the lung parenchyma, and a special note has been made of the marked inhibition of attempts at repair. In the neighbourhood of the pleura, however, this is not so, and examination of samples of tissue from regions close to but not including the pleura would lead to the conclusion that fibrous tissue is extensively laid down in the body of the lung. Actually, when the disease process involves the pleura this tissue becomes greatly thickened, and bands of fibrous tissue extend from it into the lung. Microscopic examination shows that within a short distance these bands become so branched and reduced in thickness that the fibrous network is soon inappreciable. Near the pleura, when the infection has reached the fourth stage, some very fine microscopic fields can be seen, and of the stains employed on such tissue the Drew-Murray method has given the best results. A further noteworthy observation has been that in all cases hitherto brought to notice the external surface of the pleura has been intact, and in spite of the great increase and ramification of fibrous tissue in the direction of the lung no adhesions between the parietal and visceral layers of the pleura have been noted.

The bronchial glands.—It has already been recorded that the only change in the bronchial glands that might be appreciated on macroscopic examination is some degree of enlargement, and in further consideration of the fact that this has not always been reported it may be assumed that it is not a noteworthy feature. Microscopic examination of such bronchial glands as have been attached to material

sent to the laboratory has supported this observation ; in no case has any pathological change been detected and no cryptococci have been demonstrated. It would, *a priori*, appear rash to conclude that the bronchial glands are never involved, but the fact that they have hitherto presented no pathological alterations, even in very advanced cases, is sufficiently striking to be worthy of mention.

Secondary changes.—The foregoing description is limited to the changes that are considered to result directly from the cryptococcus infection. Other changes are, however, occasionally present, but as their occurrence is uncertain and their character, in some cases obviously or in others presumably, of a secondary nature, they have been omitted from the general description. In the series of cases considered in this paper the secondary phenomena encountered have been, local passive hyperæmia, local œdema, local collapse, and vesicular emphysema, all of which are doubtless due to the peculiar distribution of pressure developed as the result of the great and irregular proliferation of the infiltrating cells.

V. Discussion.

Of the points that present themselves for discussion the most important is the identity of the causal organism concerned, since on this depends the decision as to whether the infection is directly related to epizootic lymphangitis. Unfortunately it has not been practicable to study the organism in detail ; much of the material received in the laboratory has been sent from outlying districts in preservative solution, and when fresh material has become available in Khartoum various circumstances have combined to prevent attempts at isolation in artificial culture.

In raising this question, which is that of a possible new species of cryptococcus, it must, however, be pointed out that it would be equally applicable to any case of epizootic lymphangitis in which diagnosis is made by microscopic examination alone ; in particular it might be raised in the instances in which cryptococcus infection is established in an " unusual " position in the host's body. In spite of the great variety of seats in which such infections have been recorded, only one attempt seems to have been made to create a new species, namely by Velu (1924), who reported that the cryptococcus of " lacrymal blastomycosis " in donkeys was a distinct species and named it *C. mirandei*. The grounds on which this organism was determined as a new species were its specificity for the donkey, its peculiar pathogenicity, and its ready inoculability. Examination of the supporting protocols, however, does not show that any attempt was made to transmit it to other species of equidæ or to implant it in other parts of the animal body ; nor, conversely, was any attempt made to produce a comparable infection in donkeys with *C. farciminosus*. Further, it appears—since the point was not raised—that the lacrymal cryptococcus infections that commonly occur in horses and mules are still to be considered referable to *C. farciminosus*. In

consideration of the evidence, therefore, and while not denying the *a priori* possibility of a distinct species of organism, it is concluded that at this stage there is no need to suspect the organism responsible for the pulmonary phenomena of being other than that responsible for epizootic lymphangitis, namely *Cryptococcus farciminosus*.

A further important consideration is the apparently primary nature of the lung infection. That the lung is not to be excluded as a possible seat of infection is shown by earlier records of cases in which it has occurred ; Boquet and Negre (1920) quote two such cases, but it is to be noted that in neither case was a primary pulmonary infection at issue, both being cases in which small nodules were found in the lung as the result of generalisation. It is therefore necessary to stress the observation that the lung may be a primary seat of infection, and may become infected to the most advanced degree without the existence of lesions elsewhere.

Regarding the method of infection, there is no objection to accepting inhalation as responsible. While admitting ignorance of the actual route by which inhaled organisms reach the seat of the first lesions, the climatic conditions of the Sudan, accompanied by widespread epizootic lymphangitis, certainly support the possibility.

Before abandoning the discussion of cryptococcus infection in the lungs, it is not without interest to mention two further cases encountered in the available literature. The first, by Oehl (1929), is of a case in a pig, but the infection took the form of pulmonary abscesses occurring as the result of generalised infection. The second case, in which again it has not been possible to refer to the original publication, is recorded by Fontaine, Hasse and Mitchell (1909), quoted by Park and Williams (1925). The case under consideration was one of human " systemic blastomycosis," presumably therefore generalised ; it is of interest not so much on account of the description as for an illustration (Fig. 173, p. 561 in Park and Williams) that might represent in every respect an area of equine lung in the fourth stage of *C. farciminosus* infection.

Little discussion is necessary in regard to the historical changes ; it is, however, of interest to refer to a description by Bigot and Velu (1924) of the changes that occur in the lesions of " lacrymal blastomycosis " in donkeys. Neglecting for the present the contention that in the latter condition a different cryptococcus, producing different effects, is at issue, the more complete study of the pulmonary lesions clearly shows that their conclusions require modification, both as regards the essential, as distinguished from the incidental, histological changes, and as regards the development of the parasites. It is necessary, therefore. briefly to consider their description.

In general, they describe the lesions as easily divisible into three zones : (i) a peripheral zone of dense fibrous bundles with processes which extend inwards and anastomose to enclose cavities (*vacuoles*), (ii) a middle zone of finer connective tissue bundles also enclosing spaces whose size increases proportionately to the distance from the surface, and (iii) a deep zone of very fine connective tissue strands

constituting a loose network which becomes progressively finer and ultimately vanishes. In the spaces, and particularly in the deep zone, the parasites are to be seen. Regarding the cellular changes, the peripheral zone is largely infiltrated with lymphocytes with a small proportion of fibroblasts, while in the deeper zones the more numerous cells are plasma cells (*cellules plasmatiques*). The plasma cells however, are essentially scattered, not forming any cellular mass, and not developing into giant cells. No mention is made of the parasites being anywhere intracellular, but their development is described in a manner that suggests the growth of a colony in an amorphous medium.

It is not intended to discuss this histological description at length, but in consideration of the essential changes observed in the lungs it appears that those in the lacrymal lesions are largely obscured by the intrusion of too much conjunctival connective tissue ; the description of the general arrangement might in fact almost be that of an infected area of lung in which the essential changes were being obscured by having selected material from too near the pleura. The infiltration with leucocytes, and later with " *cellules plasmatiques*," follows the lung changes with sufficient approximation ; it is, however, remarkable that giant cell formation is not only not mentioned but even denied. Whatever the actual identity of the larger mononuclear infiltrating cells, formation of giant cells is—in the lung at any rate—one of their most characteristic features.

The non-identification of giant cells is, however, explained when considering their description of the parasites. These are not represented as being intracellular, but as occurring in the form of " *plages* " consisting of large numbers of cryptococci collected into a " zoogloea " by a badly staining amorphous substance ; systematic examination is said to show such bodies of all sizes ranging from those containing only two or three cryptococci up to plasmodium-like colonies containing over a hundred. In this, the only description of the parasites, and from the microphotographs supporting it, one recognises a condition that would in the lung infection be classified as " late fourth stage." A study of the earlier stages as they occur in the lung has shown that these " *plages* " are derived from intracellular aggregates of parasites, since giant cells and mononuclear cells in various grades of distension can be seen containing cryptococci ; also many cells are to be seen in which either the actual section has failed to include nuclei or the nuclei have disappeared in the usual course of degeneration.

It seems therefore that the account given by Bigot and Velu of the changes that occur in " lacrymal blastomycosis " must be modified, since in this condition the essential changes are distorted by reason of the situation and nature of the lesion. The swelling is small, it is in a situation very rich in fibrous tissue, and is unlikely to attract clinical attention before arriving at a stage in which cellular degeneration is advanced. On this account microscopic examination will show little but fibrous reaction and parasites, the latter possibly

being still arranged in masses suggesting the outline of the cells in which they originally developed. In fact, the *essential* changes that occur in " lacrymal blastomycosis " are apparently the same as those occurring in the lung, and if this be admitted there is no more reason for considering this condition as a separate entity than for constituting *C. mirandei* a separate species. Until definite evidence to the contrary is forthcoming, both this condition and the one described in the lungs should be regarded as forms of epizootic lymphangitis and the cryptococcus at issue as *C. farciminosus*.

VI. Summary.

An account is given of a hitherto undescribed type of cryptococcus infection in equidæ, namely, a primary interstitial pneumonia.

A brief description is given of the symptoms observed during life and the gross changes observed on post-mortem examination, particular note being made of the tendency of the disease process to remain confined to the lungs.

A more detailed account is given of the pathogenesis as revealed by histological study, the essential changes being classified into four overlapping stages :—

(i) Peribronchial and perialveolar infiltration with lymphocytes.

(ii) Further and more intense infiltration with large mononuclear cells, which are presumed to be derived from wandering cells and the epithelial lining of the pulmony alveoli. This stage continues until the identity of the original tissue is lost.

(iii) Syncytia and giant cell formation, together with the first recognisable appearance of cryptococci in the monocytes and shortly afterwards in the giant cells.

(iv) Progressive destruction of new tissue by prolific multiplication of cryptococci in the invaded cells.

Especial attention is also drawn to the strong inhibition of attempts at repair.

The relationship of the causal organism to others of the same type is discussed, and it is concluded that there is no reason to regard it as other than that responsible for epizootic lymphangitis, namely, *Cryptococcus farciminosus*.

An appendix is provided comprising brief notes on the individual cases from which material has been studied.

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Appendix.

Notes on individual cases examined.

In setting out the following notes the cases are divided into two groups, the first comprising those in which cryptococci were actually identified and the second those in which either one was not prepared to identify certain suspicious bodies absolutely as cryptococci, or no bodies resembling cryptococci were seen. At the present stage the latter can only be considered as probable cases of cryptococcus infection, the basis of the provisional diagnosis being the absence of other recognisable micro-organisms and a histology conforming to the earlier stages of the proved cases. The names enclosed in brackets are of the officers who first observed the cases and provided preliminary histories.

Group I.

Cases in which Cryptococci were Identified.

Case No. 1.—(Capt. A. P. MacDonald, R.A.V.C.). Abyssinian mule, aged, Khartoum Province.

Admitted to hospital on September 4th, 1929, with a sore on the withers. Epizootic lymphangitis not suspected, but pus examined as a routine procedure and found negative for cryptococci. The wound healed under simple treatment and the mule was discharged. Three weeks later the mule was admitted to hospital with obscure respiratory symptoms, the most noticeable of which—at first at any rate—was laryngeal obstruction ; later, increasingly shallow and progressively abdominal respiration showed that the lungs were also involved.

The mule fed well throughout, but had three or four attacks of mild fever, each lasting a few days with remissions of several days in between. Actual temperature chart mislaid, but it is remembered that the temperature during the febrile periods was never very high. The mule lost condition, but not greatly.

Death occurred on October 23rd, 1929, about six weeks after first treatment for sore withers—at which time no respiratory abnormality was observed—and about three weeks from the onset of respiratory symptoms. Complete details of post-mortem examination not provided, but the abnormalities are recorded as being as follows :—

1. A " tumour " of about 2 cm. diameter on one arytenoid cartilage.
2. A chain of nodules, the largest being about 1 cm. diameter, extending down the inside of the trachea under the mucous membrane.
3. Large solid areas in both lungs—precise details not noted.

Material sent to the laboratory was a considerable portion of one lung, apparently from the dorsum, weighing about ten pounds, and devoid of trachea, main bronchi, or bronchial glands. The bulk of the tissue was greyish-white, and very tough ; pleura much thickened, but showing no adhesions. On section, inward fibrous extensions from the pleura were seen, but these soon became unrecognisable as they extended into whitish india-rubber-like tissue. Very little exudate could be obtained from the cut surface in most places ; in such areas as did provide exudate small numbers of cryptococci were visible on microscopic examination.

Histological examination of specimens from several areas of lung showed pictures that would be mainly classified as " third stage," *i.e.*, the original

lung structure was in nearly all cases entirely unrecognisable and the infiltrating monocytes and the giant cells contained large numbers of cryptococci. Very little tissue breakdown observed, and with the exception of the region of the pleura no fibrous tissue formations.

Case No. 2.—(Capt. A. P. MacDonald, R.A.V.C.). Abyssinian mule, aged, Khartoum Province.

No history at any time of wound or sore. The mule was admitted to hospital in July, 1929, suffering from a chronic cough. Various forms of treatment failed to afford any relief over a period of four months. Temperature records were at first kept, but as the mule never showed any fever they were later omitted. Ultimately the mule lost condition greatly in spite of good appetite and, with a tentative diagnosis of some form of lung tumour, it was destroyed in November, 1929.

Post-mortem showed that the lungs only were abnormal, the only observation provided being that both were extensively " solidified."

Material sent to the laboratory was a piece of lung, apparently from the dorsum, weighing three or four pounds. The outward appearance was as in Case No. 1. On section the " solidification " was seen to be less uniform, in that some areas were hard and almost dry, while others were spongy and moist, yielding appreciable amounts of purulent exudate on pressure. Microscopic examination of the exudate showed numerous cryptococci.

Histological examination of various samples showed that in different areas conditions ranging from " late second stage " to " advanced fourth stage," were present, but here again, with the exception of the vicinity of the pleura, the absence of new fibrous tissue was very noticeable.

Case No. 3.—(Capt. T. Menzies, S.V.S.). Horse, Sudan Country-bred, Darfur Province.

No history of sore at any time, the only abnormality during life being obscure pulmonary trouble. Under observation for six weeks, no diagnosis could be made and no treatment produced any relief. The horse was continuously unfit for work and was destroyed.

Post-mortem examination showed the right lung only to be abnormal, containing " a large tumour, about 10 lb. weight, in the mediastinum and invading the lung."

To analyse this note, the most striking conclusions as compared with the two earlier cases are :—

(*a*) One lung only was involved.

(*b*) The diseased area was circumscribed, although large.

(*c*) The process was associated with the hilus of the lung, although no note is made of the condition of the associated lymphatic glands.

Material sent to the laboratory consisted of a number of pieces of " tumour " in formalin solution, some being from the centre and some from the boundary area between the " tumour " and more or less normal lung. The pieces from the centre were mainly pale and dense, but showed in some cases areas comparable to caseous tubercles, ranging from inappreciable dimensions up to a diameter of three or four millimetres. The boundary between solidified and approximately normal lung appeared to be absolutely sharp in every piece of tissue from this area.

Microscopic examination showed the whole of the " tumour " to be in " advanced fourth stage," living cells being almost unrecognisable and cryptococci swarming. The caseous pseudo-tubercles proved to consist almost entirely of cryptococci in a structureless matrix, with no recognisable

remains of the cells in which they had developed. The boundary zone consisted of the inner side of recognisable lung tissue in which the only abnormality appeared to be collapse, and on the outer side of lung tissue showing considerable congestion without any alveolar or bronchial exudate. No fibrous tissue could be demonstrated in any zone, even by van Gieson's stain.

Case No. 4.—(Capt. C. P. Fisher, s.v.s.). Cyprian draught mule, aged, Khartoum Province.

The only earlier history of sore or wound was a breast-collar gall in September, 1929. Epizootic lymphangitis was not suspected, no microscopic examination was made, and the wound rapidly healed under simple treatment. The mule was regularly worked, but at some indefinite time towards the end of 1929, it began to tire in work ; later it developed laboured abdominal breathing when worked, until finally it became unworkable, and was destroyed in January, 1930. It is thus fairly certain that the lung infection was of a primary nature, and it is to be noted that symptoms were of appreciably less than four months duration.

Post-mortem examination showed that both lungs were extensively diseased, one complete lobe together with trachea and bronchial and mediastinal glands being sent to the laboratory. No abnormality was noted elsewhere.

The whole lung appeared to be involved in the sense that no considerable area of it was approximately normal ; it could only be described as having an irregularly mottled aspect with a preponderance of whitish blotches of variable size and shape. The pleura, although considerably thickened, was glistening and outwardly intact. The associated lymphatic glands appeared to be normal, or at most very slightly enlarged. Trachea and large bronchi normal.

On cutting into the lung it was evident that both as regards distribution and degree of infection the disease process was very irregular. Thick fibrous bands could be seen extending inwards from the pleura as in the first two cases, but here it was noted that even when they invaded relatively lightly infected areas of lung they soon disappeared. In the body of the lung, in the less densely affected part, thickened walls of small bronchi and thickened interlobular septa could be easily distinguished. In other parts irregular dense pale areas of all sizes and shapes were present, and in rare instances in the centre of a very dense area some purulent exudate could be expressed. This exudate was swarming with cryptococci.

Microscopic examination of a number of samples of tissue showed that every stage from " early first stage " to " late fourth stage " was present, but there was nowhere any congestion, bronchitis, or alveolar exudate, and apart from the immediate vicinity of the pleura no fibrous tissue formation. It has to be noted, however, that on examining a large number of sections, particularly of areas in the " fourth stage," a few fibroblasts were seen in the parenchyma of the lung. They were, however, very rare and could not be associated with the equally rare connective tissue fibrils that were also sometimes seen. It was from the examination of this specimen in particular that it was concluded that the absence of new fibrous tissue was referable not to absence of the tendency to form it, but rather to some kind of inhibition. Near the pleura this inhibitory influence would be less noticeable, but in the body of the lung, where fibrous tissue is normally scanty, it would be sufficient to suppress fibrous tissue formation entirely.

No abnormality of the bronchial lymphatic glands could be detected on histological examination, nor could any parasites be demonstrated in them.

Case No. 5.—(Mr. C. W. Pembrey, s.v.s.). Abyssinian mule, old, Fung Province.

The mule was presented for advice by a Sudanese native, who had no clear idea as to how long it had been sick. When first seen on February 12th, 1930, it showed rapid shallow breathing and a temperature of 103° F. Kept under observation for a fortnight it showed irregular fever, with temperature on some days normal and on others elevated, but on no occasion was it over 103° F.

Sent to the laboratory the mule was seen to be in poor condition, temperature on arrival normal, but respiration rapid, shallow,and markedly abdominal, appetite fair. Kept under observation for a month the dyspnœa became aggravated, but with the exception of one attack of fever of two days duration with temperature fluctuating between 102° and 103° F., the temperature was normal. On March 23rd, 1930, the mule fell down, and being obviously dying from asphyxia was destroyed.

Post-mortem examination, conducted with great thoroughness, showed no abnormality except in the lungs. Practically the whole of both lungs was so changed that nothing of the original parenchyma could be recognised except small bronchi, these being identified as small thick walled rings when cut. The colour throughout was greyish-white and the consistency approximately that of muscle. Along the basal edges of both lungs a strip of relatively normal lung, rarely more than two inches wide, remained. In these strips the interlobular septa were much thickened and areas of vesicular emphysema were plentiful. The pleura was thickened, but not so greatly as in the earlier cases, and entire. Bronchial glands normal.

On section, the whole of the lungs was of uniform colour and consistency, namely, greyish-white and tough. Tissue smears, after prolonged examination, showed the presence of cryptococci in very small numbers. In searching for the parasites Claudius' stain alone was employed ; the examination of other smears stained by Leishman or Giemsa showed that all recognisable cryptococci were enclosed in mononuclear cells.

Histologically, samples from several dense areas showed in every case a condition equivalent to " late second stage " with early features of the third stage. A good deal of lymphocyte infiltration was still evident, especially in the neighbourhood of the small bronchi ; in the more purely alveolar areas the cell mass consisted chiefly of monocytes, although small numbers of giant cells were also present. Cryptococci were extremely difficult to identify with certainty in the sections, but their undoubted presence in appropriately stained fresh tissue smears allows one to identify rare oval refractile bodies in slightly swollen monocytes as the causal organisms.

The relatively normal portions of lung when submitted to histological examination were found to be appreciably infiltrated and also considerably engorged with blood ; no exudate of any kind was present. In the more superficial parts vesicular emphysema was also a feature, while in the deeper layers, nearest the more intensely affected mass of lung, collapse was more evident.

Group II.

Cases in which cryptococci were either not certainly identified or were absent.

Case No. 6.—(Mr. C. W. Pembrey, s.v.s.). Abyssinian mule, aged, Fung Province.

The mule had had cutaneous lesions of epizootic lymphangitis, first diagnosed in December, 1929. It was kept for experimental treatment and

observation until June, 1930, when it was discharged as apparently cured. Within a week of discharge it was returned to hospital with respiratory symptoms typical of solidified lungs and died.

Post-mortem examination in the absence of the Veterinary Inspector was made by a Sudanese Veterinary N.C.O., who reported pale solid lungs and no other abnormality. Fresh tissue smears and pieces of preserved lungs were sent to the laboratory. Examination of the smears showed very rare bodies provisionally identified as cryptococci, but as they were in small numbers it was decided to examine the lungs histologically before giving a definite diagnosis.

Unfortunately the pieces of tissue were not well fixed, and microscopic examination showed that considerable autolysis had occurred. Nevertheless, it was possible to make out considerable peribronchial and perialveolar infiltration with a preponderance of monocytes, rare giant cells being also present. Owing to the imperfect fixation it was impossible to decide whether the alterations seen in the cells were due to cryptococcus infection or autolysis. Histological staining revealed a few unstained refractile bodies in the monocytes and the Claudius and Drew-Murray methods showed a few stained bodies that were probably cryptococci.

Considering that histologically the tissue would be classed as " second stage," it is quite probable that the bodies seen were actually cryptococci, but the generally unsatisfactory condition of the material examined makes it desirable for the present to place this case in the " doubtful " group.

Case No. 7.—(Mr. C. W. Pembrey, s.v.s.). Abyssinian mule, no details, Fung Province.

Mule brought to hospital on November 29th, 1929, respiration shallow and rapid, temperature on first examination 106° F. Blood examination negative, no superficial lesions or history of them. For four days the fever continued, but temperatures were progressively lower than at first and thereafter remained normal until the mule was destroyed on December 23rd, 1929. While under observation the mule fed fairly well, but rapidly lost condition as the dyspnœa increased ; there was never any cough.

No detailed description of post-mortem examination was provided beyond the statement that there was extensive solidification of both lungs. Pieces of preserved lung sent to the laboratory for microscopic examination were in a condition referable to " second stage " ; there was considerable lymphocytic peribronchial and monocytic perialveolar infiltration, with no congestion or exudation. No cryptococci or other organisms could be demonstrated by any method of staining.

Case No. 8.—(Mr. C. W. Pembrey, s.v.s.) Abyssinian mule, no details, Fung Province.

Admitted to hospital on January 9th, 1930, suffering from debility. Under observation for three weeks, temperature and appetite were normal and until January 28th, 1930, respiration was normal. From this date the respiration became shallower and more rapid and the mule progressively lost condition. Only one day of mild fever was observed, when the temperature in the morning was 102·6° F. Death occurred on February 10th, 1930.

On post-mortem examination the lungs only were abnormal, showing " irregular patches of hepatisation," and from these areas pieces of preserved tissue were sent to the laboratory. Naked eye examination of these determined an irregular mottled appearance, with the more solid areas almost white and the less dense areas pale pink in colour. Dense peribronchial infiltration was suggested by the prominence of the cut small bronchi.

Histologically the features were dense peribronchial infiltration, mainly with lymphycytes but with a fair number of monocytes, also an appreciable number of polynuclears. There was a certain amount of congestion and in occasional areas some catarrhal exudate in the alveoli, polynuclear leucocytes being recognisable. No fibroblastic activity and no organisms were seen.

This case is the most doubtful of those in which cryptococci could not be recognised. Most of the features conform to the earlier stages of cryptococcus infection but the congestion and catarrhal exudate are somewhat atypical. However, in consideration of the small total number of cases examined it is considered advisable to include it in the " doubtful " group since it is not yet certain that some mild catarrh may not sometimes occur.

Note.—The figures in the article are from microphotographs by Mr. A. Marshall, Wellcome Tropical Research Laboratories, Khartoum, by courtesy of the Director (Major R. G. Archibald, C.M.G., D.S.O.)

CULTIVATION OF FOOT-AND-MOUTH DISEASE VIRUS.*

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Failure to confirm the claims of Frosch and Dahmen (1924) to have cultivated the virus of foot-and-mouth disease on serum-agar have been reported (Gins, 1924, 1925; Bedson and Maitland, 1925; Stockman and Minett, 1926). Further unsuccessful attempts to cultivate the virus in modifications of media suitable for bacteria have been made by Bedson and Maitland (1925, 1927) and Olitsky and Boëz (1927). These papers, however, contain numerous observations on conditions affecting the survival of virus at temperatures suitable for growth. Burbury (1928) used a medium containing cysteine, with the object of regulating the oxidization-reduction potential, but obtained no increase of virus.

This paper records the successful cultivation of the virus in tissue-cultures. Hecke (1930, 1931[1] and [2]), independently and since our work was completed, has published his results of successful cultivation.

The Behaviour of the Virus in Tissue-Cultures of Chick Embryo.

The virus has regularly failed to grow in tissue-cultures of chick embryo. Cultures were made as follows: to 1 c.c. of minced seven-day chick embryo was added 1 c.c. of Tyrode's solution and 1 c.c. of virus. This was allowed to stand at 5° C. for 1½ hours and to 2 c.c. of the mixture was added 12 c.c. of Tyrode's solution and 6 c.c. of fresh heparanized hen plasma. The cultures were incubated in Carrel flasks, 2 c.c. in each, at 37° C. After 2 days and again after 4 days the liquid that had collected above the clot was replaced with 5 per cent. embryo extract in Tyrode's solution. The tissue grew well in all flasks. The inoculum was filtered guinea-pig vesicle fluid that was infective in a dilution of 1 in 3,000,000 when titrated by intradermal inoculation of the plantar pads of guinea-pigs. For use it was diluted 1 in 50 in $\frac{M}{50}$ phosphate *p*H 7·6 and filtered. The final dilution of vesicle fluid in the cultures was 1 in 1,500.

Thirty-three flasks were tested separately for virus after incubation for periods varying from 2 hours to 6 days. The contents of a flask were ground with sand and centrifugalized, and the supernatant fluid was titrated by the method indicated above. The potency of the virus was unchanged after 2 hours in the incubator, but after 24 hours no virus was demonstrable, nor did it reappear

* This work was carried out on behalf of the Foot-and-Mouth Disease Research Committee of the Ministry of Agriculture and Fisheries, and is reported in more detailed form in the Fourth Progress Report of the Committee, H. M. Stationery Office, London, 1931.

after incubation up to 6 days. One flask was a possible exception ; after 5 days the undiluted supernatant fluid was infective but a 1 in 10 dilution was not.

In further experiments the result was not modified by leaving the tissue and virus in contact at 5° C. for 24 hours, or by adding to the medium 1/40 volume of *Liquor Trypsinae Co.* (Allen and Hanbury). Trypsin was found (Bedson and Maitland, 1927) to prolong the survival of virus in guinea-pig serum. The rapid disappearance of the virus was not caused by alterations in reaction ; the *p*H of the medium had not appreciably changed after incubation for 24 hours. The result suggests a viricidal property of chicken tissues or serum associated with species and may be related to the fact that the chicken is not susceptible to infection with this virus.

The Cultivation of the Virus in Tissue-Cultures of Guinea-Pig Embryo.

In cultures of guinea-pig embryo the virus has been carried through 17 successive cultures and has been under cultivation from March to December, 1929. In an earlier series it had been carried through three successive cultures and had multiplied.

Technique.—The pads, lips, and tongue of guinea-pig embryos were finely minced. To about 0·66 c.c. of tissue, from 1·33 c.c. to 2 c.c. of virus was added. After this mixture had stood at 5° C. for 1½ hours, 6 c.c. of fresh heparanized guinea-pig plasma and sufficient Tyrode's solution to make the total volume 20 c.c. were added. Carrel flasks, each containing 2 c.c. of the final mixture, were incubated at 37° C. The cultures were not fed with embryo extract. In some of the later cultures hairy skin was included with the other tissues.

The first cultures (Number 1, Table I) were inoculated with a guinea-pig adapted strain of virus ' G.F." (type " O." Vallée and Carré), in the form of vesicle fluid that had been diluted 1 in 50 in $\frac{M}{50}$ phosphate *p*H 7·6 and filtered to test a culture for virus, the contents of one or more flasks were ground in sand and centrifugalized, and the supernatent fluid titrated by intradermal inoculation of a series of dilutions into the plantar pads of guinea-pigs. Up to culture number 9 (Table I) one or more flasks were tested individually on each of the first five or six days of incubation, from the 9th generation onwards on the third or fourth day only. Between each culture-passage the supernatant fluid was diluted 1 in 10 or 1 in 15 in $\frac{M}{50}$ phosphate *p*H 7·6, filtered and stored at 5° C. These filtrates were used for inoculating subcultures.

Results.—Table I summarizes some of the results of 17 generations of cultures of virus. A comparison of columns four and five shows the greatest increase of virus in any set of cultures ; in several instances the end-point of titration was not reached, but the figures

probably represent nearly the maximum potency of the virus. The titre of the virus in cultures before incubation was calculated from the titre of the culture-virus used as inoculum before it was filtered and stored and the dilution made in setting up the cultures for which it was used ; they do not allow for loss of virus during filtration and storage and tend to minimize the apparent amount of growth. It is evident, however, that the virus increased in 21 of the 25 sets of cultures ; in 5 the increase was less than one hundred fold, in 8 about one to two hundred fold, and in 8 one thousand fold or more. After passing through 17 cultures the virus was infective after having been diluted about $4{\cdot}8 \times 10^{39}$ times, filtered 16 times, and kept in culture or in storage for nine months. Comparatively little virus served to initiate growth (cultures 3, 4, 5, 11, Table I). Virus was subcultured after storage for as long as three months (culture 11, Table I). On three occasions 50 c.c. of medium was incubated in a Roux bottle and in each instance the virus increased satisfactorily (titre after incubation for four days, 1 in 1,000). The virus did not alter in virulence during cultivation ; the primary lesions were usually large vesicles and generalised lesions appeared as regularly as in infections produced by guinea-pig vesicle fluid.

Uniformity of results.—Eact set of cultures (Table I) represented from 6 to 20 flasks, and as the virus increased in some flasks from 21 of the 25 sets the results might be said to be fairly uniform. The highest titres were usually obtained after incubation for three or four days, although sometimes on the second, fifth, or sixth days. The highest titre for each of the 21 sets was also fairly constant ; it was 1 in 1,000 for six sets, 1 in 10,000 for twelve sets, and 1 in 100,000 for three sets. Even the best cultures contained much less virus than vesicle fluid from guinea-pigs, which is usually infective in a dilution of one in one million to five million.

When the results of titrating individual flasks were examined there was some lack of uniformity. One could not be certain that several flasks titrated separately at the same time would contain the same amount of virus, and it was not certain that cultures would show the greatest amount of virus on the third or fourth day of incubation, though so frequently did this occur that, in attempting to get uniform results for evaluating by comparison the effect of various modifications of technique, we adopted in the later experiments the expedient of combining several flasks for one titration and selected four days as the optimum period of incubation. This furnished more constant results both in comparing different groups of flasks from one set of cultures and in comparing one experiment with another.

The Investigation of Factors that might affect the growth of Virus in Tissue-Cultures of Guinea-pig Embryo.

The cultures described in the previous section were used as controls for concurrent experiments made to investigate the possibility of improving or simplifying the technique of culture.

The relation between growth of tissue and growth of virus.—At the time of titration of all cultures the amount of tissue growth in each flask was noted, whether absent, slight, moderate, or profuse. Some evidence was obtained from ordinary passage-cultures, the amount of tissue growth being variable in different flasks. In special experiments embryos near term were used with the expectation that their tissue would grow less vigorously; in others embryo-extract and proteose have been added to stimulate tissue growth. As a result of all these observations the definite conclusion has been reached that the amount of growth of virus does not depend upon the amount of growth of tissue. Nearly all the tissue proliferation consisted of fibroblasts; occasionally there was a small sheet of epithelial cells.

Growth of virus in embryo tissues other than pads, lips, and tongue.—The pads, lips, and tongue (mixed) were selected for the earlier cultures because it is in these tissues that lesions are found in infected guinea-pigs. M. C. Maitland (1928) had, however, shown that lesions developed on the hairy skin when it was submitted to intermittent pressure. It is also as suitable as pads, lips, and tongue for cultivation of the virus in tissue-cultures, a direct comparison having been made in two sets of cultures containing the respective tissues. As already noted, hairy skin was included with the other tissues in some of the later passage cultures.

The suitability of embryo kidney and adult kidney was tested in two experiments only, a control for comparison in each instance being made with pads, lips, and tongue. In infected guinea-pigs lesions have not been recognized in the kidney; nevertheless, a culture with embryo kidney after incubation for five days yielded as much virus as control flasks on the second, third, and fourth days. The one set of cultures made with adult kidney was unsuccessful. There is thus a suggestion that cultures of embryo kidney might promote growth of virus, but a definite conclusion is not warranted.

Modification of composition of the medium.—The effect of adding 0·5 per cent. of glucose (one experiment) and 1 per cent. (two experiments) to cultures of pads, lips, and tongue was tested by comparing the growth of virus in them with control cultures containing no glucose. Titrations indicated that the addition of glucose tended to hasten the rate of growth of virus although the yield was not greater than in the controls. It also tended to make the virus die out more rapidly; after incubation for four days the titre of the cultures containing glucose varied from less than 1 in 10 to 1 in 100, while the controls were all infective at 1 in 10,000.

In two experiments 0·75 per cent. and 2 per cent. respectively of *Liquor Trypsinae Co.* (Allen and Hanbury) was incorporated in tissue-cultures of pads, lips, and tongue and the growth of virus in them compared with controls. Trypsin appeared to accelerate the rate of growth of virus but, unlike glucose, did not cause it to die so rapidly.

The addition of 1 per cent. (two experiments) and 2 per cent. (one experiment) of proteose was tested in a similar manner. The

proteose was kindly supplied by Mr. E. N. Willmer of the Department of Physiology who made it from Witte's peptone ; it was found by him to stimulate the growth of chick embryo in cultures and was added to our cultures for this purpose as already noted. Of 18 pairs of flasks (one of each pair being a control) examined after incubation for one to eight days, in eight the virus was stronger in those containing proteose, in seven the virus was equal in each, and in three there was more virus in the controls. There was thus a suggestion that the addition of proteose may have increased the yield of virus, but this did not depend on increased tissue growth. In cultures containing proteose digestion of the clot began earlier than in the controls ; usually it was detached from the glass after 24 hours.

TABLE I.

SUMMARY OF 17 GENERATIONS OF VIRUS CULTURES, SHOWING THE AMOUNT OF GROWTH.

Number of culture	*Duration of storage of culture virus used as inoculum.*	*Estimated titre of virus in the cultures before incubation*	*Highest titre of the virus in any flask after incubation*
1	14 days	1/460,00	>1/100,000
2	7 days	>1/660	>1/10,000
3	1 day	>1/4	>1/1,000
4	13–14 days	>1/6	>1/10,000
5	5 days	1/6	>1/10,000
5*a* *	4– 5 days	>1/60	>1/100,000
6	5– 6 days	1/60	>1/10,000
7	7– 8 days	1/60	neg.
7*a*	12–13 days	1/60	1/1,000
8	8– 9 days	1/10	1/1,000
8*a*	14–15 days	1/10	1/1,000
9	6– 7 days	1/10	>1/10,000
9*a*	13–14 days	1/10	>1/10,000
10	3– 5 days	1/10	1/10
10*a*	4 days	1/10	>1/10,000
11	3 months	>1/4	>1/10,000
12	4– 5 days	>1/100	>1/1,000
12*a*	12–13 days	>1/100	>1/10,000
13	3 days	>1/60	neg.
13*a*	7 days	>1/60	>1/10,000
14	6 days	>1/130	1/100
14*a*	13 days	>1/100	1/1,000
15	4 days	1/10	1/100,000
16	3 days	1/100	1/10,000
17	6 days	1/100	1/10,000

>=not an end point.

* 5*a* &c., indicates a second set of cultures of the 5th, &c. passage.

Experiments on survival of the virus in media designed to simplify the method of culture.

Nutritive media which favour the survival of virus, i.e., the total time during which any virus can be demonstrated at temperatures suitable for cultivation, are not necessarily those which favour or even permit its growth. Modifications of media which promote survival may not therefore always be successful when tested by daily titrations of cultures to determine an actual increase or growth curve of virus. This method of experiment nevertheless has its place, as conditions

not suitable for survival may be detected by simple experiments. In this section some observations on survival are recorded, and in a few instances conditions that favoured survival have been submitted to the test of daily titrations.

Virus survived as long (eleven days) in cultures of adult guinea-pig kidney as in cultures of embryo pads, lips and tongue, or hairy skin. The clot around the pieces of kidney began to disintegrate within 24 hours and in three days it was completely dissolved. In a medium composed of minced adult kidney, serum, and Tyrode's solution, in the proportions used for the tissue-cultures, the virus also survived for eleven days. This medium has since been used for the cultivation of vaccinia virus (Maitland and Maitland, 1928) as well as other viruses. Foot-and-mouth virus, after three passages in the medium, having been nine days in the incubator and filtered twice, was still infective, but further subcultures were not obtained. There was no certainty that the virus had multiplied, but neither was it certain that the presence of virus in the third culture was due merely to survival of the original inoculum, which in making the cultures had been diluted almost to its original infective titre. Substituting minced guinea-pig embryo was not successful, likewise increasing the proportion of kidney or of serum. Adult liver markedly shortened survival. The addition of trypsin, and incubating the medium in an atmosphere of hydrogen, carbon dioxide, or nitrogen, did not promote survival.

The survival of virus in this medium appeared to depend upon the persistence of some function or functions of the cells of the tissue. Heating the tissue at 100° C. for 30 minutes reduced the period of survival of virus to less than three days. Heating at 45° C. for 15 minutes resulted in survival for five days, and suggested that this amount of preliminary heating had hastened the loss which permitted the virus to survive. The same conclusion was reached through experiments in which the medium was incubated three, four, and five days before the addition of virus. The longer the duration of the preliminary incubation of the medium, the shorter was the period of survival of the virus in it.

Immunity of Guinea-pigs after recovery from infection with culture virus to "G.F." virus.

Four guinea-pigs that had recovered from infection with virus from culture eight (Table I) were tested for immunity one month after the date of infection by an intramuscular injection of guinea-pig vesicle fluid. Although they received more than an infecting dose for normal animals, none developed any lesions. It was thus evident that recovery from infection with culture-virus was associated with immunity towards the strain of virus from which the culture-virus had originated.

Discussion.

The experiments of Hecke (1930, 1931[1] and [2]) and our own are mutually confirmatory in several essential respects, although his

methods of cultivation were somewhat different. He worked throughout with small amounts, the total bulk of one culture being about 0·008 c.c. He found that chick embryo was not suitable, and succeeded with tissue-cultures of skin from guinea-pig embryos in carrying the virus through 11, 12, 14, and 17 passages on different occasions, the total period of incubation being for the first two 49 and 46 days at 37° C. and for the last two 59 and 81 days at 30° C. Further subcultures were in each series unsuccessful. The maximum increase of virus in a single culture was about one thousand-fold, and the highest titres were obtained at 37° C., after incubation for two to three days (1 in 1,000 to 1 in 10,000). The greatest total increase of virus throughout any one series of passage cultures was about 2×10^{20}. A later series was still under cultivation after a total incubation of 100 days.

The so-called " loss of virulence " of the virus in culture ascribed by Hecke as the cause of the failure to continue subcultures was not met with by us. It is an open question whether his subcultures finally came to an end because the virus became avirulent and therefore could not be detected or whether it merely failed to grow. At the time when our cultures were voluntarily discontinued the strain of virus we had used still caused typical infection in guinea-pigs.

Hecke cultivated the virus in tissue-cultures of guinea-pig testis from young animals through eight passages at 30° C. but considered this medium to be less suitable than tissue-cultures of embryo skin because he was only able to carry the virus through a small number of subcultures. The virus did not grow in cultures of adult guinea-pig liver or parotid, or in cultures of foetal small-intestine. He also was of opinion that the increase of virus in the cultures did not depend on the amount of tissue growth.

The method of cultivation described in this paper has certain obvious advantages. It is easier to work with larger amounts, flasks are less liable to bacterial contamination than small drop-cultures on slides, and titration is more readily made with larger amounts of culture-material. The few successful cultures in Roux bottles suggests that the production of culture-virus in amounts large enough for the production of vaccine for general use may eventually be possible.

Summary.

1. The virus of foot-and-mouth disease rapidly became non-infective in tissue-cultures of chick embryo tissue in hen plasma. This was not overcome by the addition of trypsin to the medium, nor was it related to changes in *p*H. It was probably evidence of a species peculiarity of chicken tissue and plasma.

2. The virus has been cultivated through 17 successive cultures, in cultures of guinea-pig embryo tissue in clotted guinea-pig plasma. The tissues used were pads, lips, and tongue. Hairy skin appeared to be as satisfactory. Cultures of embryo kidney have in one instance permitted growth of the virus.

3. Cultivation did not decrease the severity of the infection produced by the virus.

4. Recovery from infection produced with virus from the eighth culture was associated with immunity against the strain of guinea-pig adapted virus from which the culture-virus originated.

5. The amount of growth of virus in individual flasks was irregular. The maximum occurred most frequently after incubation for three and four days.

6. Comparatively small amounts of virus served to initiate growth ; an amount of virus in the cultures before incubation estimated as an infective titre of one in four sufficed.

7. Virus has been subcultured after storage for three months at $\pm 5°$ C.

8. The virus frequently increased 1,000 times or more during three to four days incubation ; on one occasion it multiplied at least 10,000 times.

9. Satisfactory cultures have been made by incubating 50 c.c. of medium in a Roux bottle.

10. The highest titre of virus observed in cultures was 1 in 100,000.

11. There was no relation between the amount of growth of tissue in the cultures and the growth of virus.

12. The addition of proteose to tissue-cultures probably increased the amount of growth of virus.

13. There was some evidence that the addition of glucose or trypsin to the medium might accelerate the rate of growth of the virus.

14. Growth of the virus was not observed in tissue cultures of adult guinea-pig kidney, although the virus survived as long in this medium as in cultures of embryo tissues in which it multiplied.

15. Growth of the virus was not certainly obtained in a medium consisting of minced adult guinea-pig kidney in serum and Tyrode's solution although the virus survived as long in this medium as in tissue-cultures in which it multiplied. This medium promotes the growth of vaccinia and some other viruses. Attempts were made to grow the virus in modifications of the medium without success. The survival of the virus in it appeared to depend on the survival of one or more undetermined functions of the tissue cells.

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STUDIES ON BOVINE MASTITIS.

IV. The Occurrence in Mastitis of Hæmolytic Streptococci showing Group Similarity to *Streptococcus Pyogenes* of Man

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In a preliminary report, published in 1929, on the bacteriology of 113 clinical cases of mastitis it was stated that 82 were caused by streptococci. In 52 of these cases the streptococci belonged to the group referred to by J. H. Brown (1919) as beta-hæmolytic.* The characters of most of these 52 strains displayed a broad correspondence, bringing them into line with a group of mastitis streptococci which has been described by earlier authors under the name *Streptococcus mastitidis*. At the time of this preliminary examination it was noticed that certain strains were exceptional in some of their properties, *e.g.*, hæmolysin production and virulence, but sufficient work had not been done to make clear the nature of these differences. The purpose of the present article is to describe more fully these exceptional strains and to present cultural and serological evidence which indicates that as a class they are indistinguishable from strains of direct human origin belonging to the *Streptococcus pyogenes* group.

Source of the Exceptional Strains.

A more exhaustive examination of the original strains has shown that 13 of them call for special attention in this paper. These are designated S21, 34, 43, 50, 55, 70, 75, 88, 89, 90, 91, 93 and 38. Each strain was derived from a different cow belonging to a different herd, except S88, 89, 90 and 91, which were from four cows in one herd.

For the histories of the cows and for other details we are greatly indebted to the following veterinary surgeons, Messrs. R. F. Montgomery and W. Hall Savage, H. Tudor Hughes, F. Beckett, A. R. Smythe, R. L. Phillips, J. M. Armfield, C. A. Powell, J. R. Hewer, D. S. Rabagliati, and W. T. MacGregor.

The information obtained was briefly as follows: Six of the cows had had at least three calves, two cows were described as "aged," and with the remainder the age was not stated. In nine animals symptoms of mastitis appeared within fifteen days after calving and in six of these within the first seven days. The cases were not confined to any one period of the year, five occurring between May and August and eight between October and February. In four animals, a single quarter was involved, two animals were affected in two quarters,

* After this paper had been written our attention was directed to an article by Haupt (Zentralblatt f. Bakt. Orig. 1931, **120**, 291), in which it is concluded that many of these bovine streptococci are not beta-hæmolytic but belong to Brown's alpha-hæmolytic group. It is proposed to reply to Haupt's criticism in an early issue of the Zentralblatt.

two in three quarters, and two in all four quarters. In four cases, it was stated that the mastitis was accompanied by systemic symptoms. All the cows were milked by hand, but there was no history of illness among the milkers which could be associated with the mastitis. In every instance the udder secretion was grossly altered in appearance, resembling whey and containing masses of flocculent material. Microscopic examination usually showed large numbers of small cocci, arranged as diplococci or short chains. With S75 many of the cocci were encapsuled. The secretion in 0·1 to 1·0 c.c. amounts was plated in deep ox blood agar, and beta-hæmolytic colonies which had developed after 48 hours incubation were counted.

It may be assumed that in most of the cases the streptococci in question were the cause of the disease since they were present in the secretion in large and preponderating numbers (from one to eight million per c.c.) Thus in five cases the appearance of the plates suggested that all the colonies were of the same kind, in two cases other streptococci were present as well, and in two cases *B. pyogenes*.

The following streptococcus cultures have been examined for comparison.

(*a*) Forty-two cultures of *S. pyogenes* of direct human origin. Most of these were obtained from Dr. F. Griffith and had been isolated from cases of scarlet fever, puerperal septicæmia, and human throat infections. Six were isolated in 1930 by Dr. W. M. Scott from throat swabs of patients in a milk-borne outbreak of sore throat in this country.

(*b*) Three cultures isolated from the cow's udder and regarded by the workers responsible as of human origin :—

(i) " *Uphoff*," a strain of *Streptococcus epidemicus* (Davis, 1912), received through the kindness of Dr. F. B. Hadley, University of Wisconsin.

(ii) " 1452," a scarlet fever strain isolated by Jones and Little (1928) and sent by Dr. Hadley.

(iii) " *Strain IIIE*," isolated from a case of mastitis by Dr. K. Diernhofer, Veterinary High School, Vienna, and kindly forwarded by him.

(*c*) Eleven beta-hæmolytic cultures chosen from a large series as representing typical mastitis streptococci, including two cultures, S81 and 84, which were recognised to be different serologically.

Methods.

Uniform methods were used throughout.

(1) The characters of the growth in meat infusion broth *p*H 7·4, were noted after 18 to 24 hours at 37° C. A smear of the deposit was fixed by heat and stained with 1 per cent. thionin blue in 5 per cent. phenol.

(2) The morphological appearance of colonies on 5 per cent. ox blood agar plates was observed after 24 and 48 hours at 37° C. and again after the plates had been standing at laboratory temperature for several days.

(3) 24-hour cultures in 5 per cent. ox blood broth were examined for capsules by means of Indian ink.

(4) The effect upon skimmed milk tinted with litmus was observed during five days incubation at 37° C., 5·0 c.c. of the medium in 13 × 1·3 cm. tubes being sown with two drops of 18 hour serum broth culture.

(5) Note was taken of the *p*H attained after five days incubation in infusion broth, *p*H 7·4, containing 1·0 per cent. glucose (Avery and Cullen, 1919), lactose, and saccharose respectively, care being taken to avoid an excess of indicator solution. The ability of strains to produce acid from salicin, mannite, inulin, and raffinose was also tested.

(6) The capacity of strains to hydrolyse sodium huppurate to glycin and benzoic acid was tested by cultivation in infusion broth containing 1·0 per cent. hippurate (Ayers and Rupp, 1922). After five days at 37° C., amounts varying from about 0·2 to 0·3 c.c. 12 per cent. ferric chloride solution containing 2·5 c.c. concentrated HCl per litre was added to 1 c.c. clear supernatant, and *immediately* mixed. In this test attention has to be paid to the amount of ferric chloride used, since this reagent precipitates both benzoic acid and hippuric acid, as well as protein. The hippurate precipitate, however, is soluble in a concentration of iron solution in which ferric benzoate is still insoluble. Especially when using a fresh batch of medium, it is therefore necessary to make preliminary trials to determine the amount of iron solution which gives good clearing with the unsown medium.

(7) The hæmolytic activity of strains was examined by cultivation for seven hours in 15 per cent. horse serum broth, and by surface and deep cultivation in plates of agar containing 5·0 per cent. defibrinated ox blood. The agar was poured to a depth of about 2 mm.

The liquid medium was warmed to body temperature before being sown from a warm 18-hour serum broth culture. Under these conditions an experiment with a few strains showed that maximum lysin production occurred after seven to eight hours incubation. The medium was centrifuged and falling amounts of the supernatant fluid from 1·0 c.c. to 0·001 c.c. were mixed with 0·9 c.c. 1·0 per cent. washed ox red cells, dilutions being made with unsown medium. Tubes were examined after water-bath incubation for two hours at 37° C., and again after standing at room temperature overnight. The smallest amount of supernatant fluid causing complete hæmolysis under these conditions was taken as the M.H.D.

In the agar medium the extent of hæmolysis around several well-isolated colonies was observed after 48 hours incubation. The results were expressed in terms of the diameter of the hæmolytic zone, and of the ratio between the diameter of the colony and of the zone.

(8) Tolerance to bile was tested by surface cultivation on sheep blood agar containing 10 per cent. and 40 per cent. ox bile respectively.

(9) Toxin production was tested by cultivation for four days at 37° C. in 1·0 per cent. horse serum broth, after which the culture was centrifuged and the supernatant fluid passed through a Seitz filter. The filtrate was tested by injecting intradermally 0·1 c.c. 1 in 100 dilution. All injections were made on an adult known to be susceptible to scarlet fever toxin.

(10) Pathogenicity tests, using 24-hour serum broth cultures, were made on rabbits intravenously and on mice and guinea-pigs intraperitoneally. Mice were inoculated in pairs with increasing dilutions of culture starting with 0·5 c.c. In a few instances the capacity to cause mastitis was tested by injecting the culture into the udder through the teat canal.

(11) Two serological methods were employed, (*a*) a rapid slide-agglutination test, using a thick bacterial emulsion of uniform density ; (*b*) a precipitin

test, similar to that of Lancefield (1928), using an extract prepared by treating the deposit from a 24-hour glucose broth culture with N/20 HCl at 98° C. for 15 minutes. These methods are found to give parallel results. Sera, obtained from rabbits or goats which had received a series of injections of heat-killed streptococci, were rendered group or type-specific by preliminary treatment with selected heterologous strains. Results obtained by direct precipitation or agglutination were confirmed by specific adsorption.

Characters of Strains.

A summary of the more important characters detailed below is given in Table I.

(*Note.*—For the sake of brevity, strains will be described in the following way : those of the S21 series as " S21 strains," *S. pyogenes* strains of direct human origin as " human strains," and the representative mastitis cultures as " udder strains ").

Growth in broth.—Growth in this medium was without differential value, appearing as a flocculent deposit leaving the supernatant fluid clear or very slightly turbid. S21, S50, and a few human strains produced a slight and uniformly turbid growth with practically no sediment. As a rule, S21 strains could be distinguished from udder strains by the relatively smaller size and more rounded appearance of the cocci and the chains were usually shorter. Udder streptococci tend to grow in longer chains, and the individual elements are larger and often flattened transversely.

Appearance of Colonies.—Colonies of the S21 strains were small, hemispherical, and glossy, and by transmitted light were seen to be finely granular. In some cases, the colonies, instead of being truly hemispherical, were somewhat flattened at the periphery and slightly less glossy. By contrast, colonies of the udder strains were as a rule relatively large and rather more granular by transmitted light. Dr. F. Griffith, to whom we submitted S21, 88, 38, and 70, agreed that by the appearance of colonies these strains could not be excluded from the S. *pyogenes* group.

Capsules.—All the S21 strains, strain " Uphoff," and nine of the human strains, including those from patients in the milk-borne epidemic, were examined after they had been in stock for some time, but no distinct capsules could be made out.

Fermentation Reactions.—Lactose was found to be superior to glucose for differential purposes. Three human strains produced very slight change in lactose broth, the *p*H not falling below 6·6. Of the remainder, seven gave a final *p*H between 5·5 and 6·0, with 30 the final *p*H lay between 5·1 and 5·4, and with two it was *p*H 5·0. In the S21 series, S38 gave a final *p*H 6·6 to 6·8, with S70 the endpoint was 5·5 to 5·7, and with the remaining eleven strains the final *p*H fell between 5·0 and 5·2. In contrast, ten udder strains gave a final *p*H below 5·0 and one had lost the power of attacking lactose.

With glucose, eleven out of 42 human strains gave a final *p*H of 4·6 to 4·9, with the remainder it was 5·0 or above. In the S21 series,

TABLE I.

CHARACTERS OF STREPTOCOCCUS STRAINS.

Strains.	Total No. examined.	Final pH in lactose broth.	Hydrolysis of sod. hippurate.	Litmus milk.	Hæmolysis.			Growth on blood agar containing.		Toxin.	Pathogenicity (M.L.D.)	
					Deep col. in Blood Agar.							
					Col./zone ratio.	Diameter of zone in mm.	M.H.D.	10% bile.	40% bile		Mouse.	Rabbit.
Bovine	11 (usually)	<5·0(10) 7·0 (1)	+(11)	AC2(10) —(1)	1 : 2 to 1 : 3	(9) 2 or less	·1(2) 1·0(4) >1·0(5)	+ + + (11)	+ +	—(21)	·5 or over	>2·5 to 5·0
S21 series	13	6·6(1) 5·5(1) 5·0-5·2 (11)	—(13)	A5*(12) —(1)	1 : 6 to 1 : 9	(13) up to 4·5	·01(12) ·1(1)	+ (13)—		—(12) + +(1)	·000005(1) ·0005(2) ·0005(2) ·005(5) ·05(2)	±0·1
Human	42	6·6(3) 5·5-6·0 (7) 5·1-5·4 (30) 5·0(2)	—(42)	—(3) A5*(28) AC2 to 3 (11)	1 : 5 or below	(42) 2 to 4	·01(11) ·1(31)	± (39) — + + + (3)	+ +	.....	.....	.....

Note.—Figures in brackets refer to the number of strains exhibiting the particular character. The + and — are self-explanatory ; ..... means not examined.

* Some of these strains produced a soft clot within the five-day period.

the endpoint with all strains except one was about *p*H 4·8. The udder strains gave a final *p*H 4·8 or below, usually about *p*H 4·6.

Saccharose was fermented by all the human and S21 strains and, with one exception, by all the udder strains. Salicin was fermented by all the human strains save one, and by all the S21 strains. Of the udder strains, six fermented salicin, two failed to do so, and three showed slight fermentation. The only strains fermenting mannite were three human strains. No strains fermented inulin or raffinose.

Litmus milk.—The effect of strains upon this medium was broadly parallel with their effect upon lactose. All the udder strains, with the exception of the one which did not attack lactose, strongly acidified the medium and produced within 48 hours firm coagulation with some reduction of litmus in the lower part of the tube.

The three human strains which had a slight effect only on lactose in broth produced extremely slight acidity in litmus milk. Three-fourths of the remaining human strains and all those in the S21 series rendered litmus milk acid within five days, with or without a soft clot. The rest of the human strains produced acid and clot on the second or third day.

Hydrolysis of sodium hippurate.—The eleven udder strains used as controls hydrolysed hippurate. By contrast, all the human and the S21 strains failed to split hippurate.

In order to ascertain whether in positive cases the whole of the available hippurate was hydrolysed and whether in negative cases any of this substance was attacked, 19 cultures, of which seven had been read as positive and twelve as negative, were kindly examined for us by Dr. G. W. Clough, of this College.

The results showed that in cultures regarded as positive the whole of the sodium hippurate had been replaced by an equivalent amount of benzoic acid, while from cultures regarded as negative the whole of the hippurate originally present could be recovered in the form of hippuric acid.

In our hands, therefore, the hippurate test has given results as highly satisfactory as those obtained by Ayers and Rupp.

Hæmolysis.—In the S21 series, the M.H.D. in twelve cases was 0·01 c.c., and in one case 0·1 c.c. With 42 human strains, the M.H.D. in twelve cases was 0·01 c.c., and in one case 0·1 c.c. With 42 human strains, the M.H.D. was 0·01 c.c. with eleven and 0·1 c.c. with 31. With these amounts hæmolysis was complete, but in both series there was frequently considerable lysis with smaller amounts of culture fluid. The udder strains produced only small amounts of hæmolysin ; with two strains the M.H.D. was 0·1 c.c., with four it reached 1·0 c.c., and with five strains there was no hæmolysis with 1·0 of culture fluid. Three of the latter, however, were grown in broth over a deposit of red cells and found to produce some degree of hæmolysis.

Hæmolysin production in the liquid medium was broadly parallel to that shown by colonies growing in or on ox blood agar. Deep

colonies of the S21 strains were mostly small and surrounded by clear zones up to about 4·5 mm. in diameter, the colony/zone ratio being from 1 : 6 to 1 : 9. In the larger series of human strains colony size and hæmolytic properties were more variable. With some the hæmolytic zones were less than 2·5 mm. in diameter, while others measured as much as 4 mm. The colony/zone ratio rarely exceeded 1 : 5. With the udder strains deep colonies were as a rule relatively large and the zones though distinct were narrow, usually measuring less than 2 mm. The colony/zone ratio was in the region of 1 : 2 or 1 : 3.

Similar differences could be observed where surface colonies on blood agar were examined. With the udder strains the zone about well-separated surface colonies was often greenish, and no doubt in the past strains such as these have often been referred to as *Streptococcus viridans*.

Effect of Bile.—The udder strains grew well on sheep blood agar containing 10 per cent. ox bile, and almost equally well when the medium contained 40 per cent. bile. With the S21 series growth was meagre with 10 per cent. and usually absent with 40 per cent. bile. With the human strains growth was generally absent or extremely slight with 10 per cent. bile and was inhibited in nearly all cases by 40 per cent. bile. Three human strains gave fairly good growth even in the presence of 40 per cent. bile.

Toxin Production.—Altogether, 21 udder strains have been tested but none was found to possess definite toxic properties.

In the S21 series the only strain which produced demonstrable toxin was S70. Culture filtrates of this strain, diluted 1 in 100, caused on two occasions reactions measuring about 17 mm. in diameter 20 hours after injection. To determine the relationship of S70 toxin to that of scarlet fever, skin neutralisation tests were made with scarlet fever antitoxin (concentrated globulins) received from the Wellcome Research Laboratories through the kindness of Dr. R. A. O'Brien. Dilutions of S70 toxin and comparable dilutions of Dochez toxin (No. 1892, National Collection of Type Cultures), corresponding in each case to about two skin test doses, were mixed with equal volumes of antitoxin diluted at 1 in 100 and 1 in 400. After incubation at 37° C. for an hour, 0·1 c.c. amounts of each mixture and of toxin controls were injected intradermally. The results pointed to some resemblance between the two toxins, since the Dochez toxin was neutralised by antitoxin almost completely at 1 in 800 and completely at 1 in 200, while the S70 toxin was neutralised slightly at 1 in 800 and partially at 1 in 200.

Pathogenic Effects.—It is well known that mastitis streptococci are of low virulence for laboratory animals. In our experience also, mice usually tolerate amounts as large as 0·5 c.c. serum broth culture intraperitoneally and rabbits survive intravenous inoculations of 2·5 to 5·0 c.c.

On the other hand, most of the S21 strains were highly virulent for mice, the minimum fatal doses being approximately as follows :

0·05 c.c. with two, 0·005 c.c. with five, 0·0005 c.c. with two, 0·00005 c.c. with three, and 0·000005 c.c. with one. In each of these cases, the two mice inoculated died within seven days with streptococci in the heart blood. Rabbits showed much variation in susceptibility, and no attempt was made to determine with the same precision the minimum fatal doses for these animals. However, with five strains 0·1 c.c. culture was sufficient to cause death, with five 0·01 c.c. was sufficient, and an occasional rabbit died after receiving 0·001 c.c.

Six strains were tested on guinea-pigs in doses of 1·0 c.c. and 0·1 c.c., with the result that one strain, S93, with the larger dose caused death from acute peritonitis.

No systematic investigation has been made of the mastitis-producing power of strains when injected into the udder by the teat canal or other route. S89, however, in a dose of 1·0 c.c. serum broth culture was injected by the teat canal into one quarter of the udder of a lactating cow immediately after milking. This caused an acute mastitis followed by rapid atrophy of the quarter. The secretion was whey-like and flocculent, and after the 30th day no more could be obtained. During this time the secretion was plated daily, and the streptococcus count was never observed to fall below one million per c.c. and on several occasions it rose to at least one hundred millions per c.c.

A second lactating cow was injected in a similar manner with 1·0 c.c. serum broth culture of a typical udder strain, freshly isolated from a chronic case of mastitis. This caused a mastitis which was initially less severe than in the first case and after a few days symptoms became quiescent. Although the milk yield was diminished during the first fourteen days, the characters of the secretion were only slightly altered. The milk was examined culturally once daily or every other day and, though streptococci were present on all occasions, the count was much lower than in the former case, being almost invariably less than one million per c.c., and as a rule, below one hundred thousand per c.c. The result of this experiment, however, cannot be compared strictly with the first one, because streptococci had been previously injected into another quarter of this cow, and it is possible that the mastitis resulting from this inoculation may have influenced the response to the second injection.

Serological Characters.—A full account of work which has been undertaken by one of us (A.W.S.) on the serological classification of mastitis streptococci will be published later. Only work which has a direct bearing on the present problem will be given now.

Of ten udder strains concerned in this report, eight belonged to one antigenic type (type I) and two (S81 and 84) were of other types. S81 showed group similarity to a type strain of Griffith's scarlet fever group III (1927), although reciprocal adsorption indicated that the likeness was not specific.

The S21 strains and strain IIIE (Diernhofer) were entirely different antigenically from the udder strains and, with the exception

of S38 and S70, showed common group characters. Apart from these two strains, however, the group contains at least three specific types, of which S21, S90, and probably S43 are representative.

The S21 strains were also tested against sera prepared with six streptococcus strains of human origin, regarded by Griffith as representing the most common types of the *S. pyogenes* group. The six strains selected included one from each of Griffith's serological scarlet fever types, 1, 2, 3 and 4. Group agglutination was obtained in some cases, particularly with scarlet fever type 4 serum, but no specific likeness was observed. Further, no specific likeness could be observed when strains of human origin, representative of each of the *S. pyogenes* types so far recognised by Griffith, were tested against sera prepared by the use of strains of the S21 and udder series. Group similarity, however, was commonly observed, particularly with S90 serum and less often with S21 and S55 sera.

Thus, it is evident that there is a group similarity between the S21 strains and *S. pyogenes* of human origin, while both groups are quite distinct antigenically from the udder strains.

These results have been confirmed in part by Dr. Griffith, who examined S21, 88, 38 and 70. S88 was found to be too granular for agglutination tests with sera prepared with strains of *S. pyogenes* from man. Type-specific agglutination was not obtained with any specific serum possessed by Dr. Griffith, but this was not considered surprising in view of the numerous *S. pyogenes* types which exist. S70, the weak toxin producer, could not be identified as belonging to any one of Griffith's *S. pyogenes* types 1, 2, 3 and 4, which include 70 per cent. of streptococcus strains associated with epidemic scarlet fever. Dr. Griffith has pointed out to us, however, that many strains of *S. pyogenes* which produce small amounts of toxin are capable of eliciting a rash in very susceptible people. S70 may, therefore, be held to be a strain of this nature.

Discussion.

Although the S21 strains and those selected from a large number as representing typical udder strains fall into the group defined by J. H. Brown as beta-hæmolytic, it is clear that the two groups differ widely in their characters. It is also clear, both from our own and from independent evidence, that the S21 strains show a close group similarity to those of *Streptococcus pyogenes* from man. This naturally suggests that these streptococci had been implanted in the udder from a human source, probably from the milker. The evidence as it stands, however, does not entirely rule out the possibility that they are bovine strains, derived perhaps from some other part of the animal, but if so, it must be held surprising that similar streptococci have not been encountered more frequently. From the experimental standpoint, the view that the S21 strains are of human origin is supported by the knowledge that very small numbers of hæmolytic streptococci from man are capable of producing mastitis when

inoculated by way of the teat (Jones and Little, 1928), or when cultures are applied to an abrasion at the teat meatus (Davis and Capps, 1914). There is also the knowledge (*vide infra*) that under natural conditions streptococci of human origin do occasionally invade the udder.

A point which might be held to contra-indicate the human origin of the S21 strains is the absence of any definite history of disease among persons in contact with the cases. It may be remarked, however, that, even when the udder is infected with streptococci which there is good reason to regard as dangerous to human beings, disease among persons in contact does not invariably occur. There will be no question as to the abundant opportunity for infection of the udder with streptococci from man in hand-milked cows, and hæmolytic streptococci are by no means uncommon in the throats of people who show no apparent ill-health.

In the present series of fifty-two cases of clinical mastitis which were associated with hæmolytic streptococci, strains of the S21 group were met with in no less than thirteen. It ought to be stated, however, that these thirteen cases were probably among the most severe clinically, a point which should be borne in mind when the relative incidence of such streptococci is considered. Apart from actual clinical cases, strains of the S21 group have not yet been encountered.

Organisms with the characters of *S. pyogenes* from man have been cultivated from the cow's udder on previous occasions. In the earlier work there are instances where streptococci with unusual characters have been isolated, some of which might well have been shown to belong to this group if suitable differential methods had been available. A case in point is that of Savage (1906-8), who isolated from two cases of mastitis and from a case of teat ulceration streptococci of high virulence for mice.

Davis (1912) described under the name of *Streptococcus epidemicus* a virulent streptococcus cultivated from the throats of patients in the Chicago milk-borne sore throat epidemic of 1911. The organism was looked upon as a member of the *S. pyogenes* group with special characters. When freshly isolated the streptococci are often encapsulated and, in consequence, young colonies on solid media incubated in a moist atmosphere have a watery appearance and tend to run together. Since 1912 streptococci with these characters have been cultivated frequently from persons with sore throat in milk-borne epidemics and in a number of instances from the milk of cows associated with such epidemics. The organism has also been isolated on 15 occasions from the milk of cows in the absence of epidemics by Frost, Thomas, Gumm and Hadley (1930), whose work pointed to a rather wide distribution of the organism among cows.

Jones and Little (1928) cultivated scarlet fever streptococci (Strain 1452) from a case of mastitis in a cow belonging to a small herd the milk of which was associated with an outbreak of scarlet fever. The toxin produced by this streptococcus was neutralised by scarlet fever antitoxin. From a case of mastitis in another herd, unassociated with human disease, a streptococcus was recovered which by Lancefield's precipitin technique proved to be similar antigenically to strain 1452 and to two known scarlet

fever strains. We have found that the toxin of strain 1452 is neutralised by Wellcome scarlet fever antitoxin almost as effectively as Dochez (NCTC 1892) toxin.

Diernhofer (1930) isolated from a cow with chronic udder catarrh a hæmolytic streptococcus of high virulence, referred to as " Strain IIIE," which was recognised as closely related to *S. pyogenes* from man. We have found this organism to be indistinguishable from certain of the S21 strains.

Seeleman and Hadenfeldt (1930) isolated streptococci similar to *S. pyogenes* from man from 14 cases of mastitis which occurred within a period of a few weeks in a herd of 80 cows. The cases were severe and accompanied by general symptoms. There was no history of illness among the milkers, though several of them had consumed the milk and from the throats of two of them similar organisms were recovered. The description of the streptococci isolated by these authors makes it clear that they were similar to those of the S21 series.

J. Smith (1929) made a bacteriological examination of udder secretion from 25 clinical cases of mastitis, and from eight of them isolated actively hæmolytic streptococci. Culturally and serologically, these organisms could not be distinguished from hæmolytic streptococci from human and certain other animal sources. Four of them produced weak toxin which was neutralised by scarlet fever antitoxin. The cow strains were regarded as probably of human origin in the first place—in fact, in two cases the evidence strongly suggested that the animals were the source of an outbreak of tonsilitis in man. One of the strains isolated by Smith was examined by Downie (1930), who found it to be highly virulent for mice and rabbits and related serologically to a streptococcus from a case of erysipelas. In Smith's work surface isolation on blood agar plates was apparently used, so that in selecting hæmolytic colonies strains showing narrow beta-zones would probably be excluded. This point ought to be borne in mind when considering Smith's conclusion that it is not possible to distinguish hæmolytic strains from human and animal sources. Evidently the hæmolytic mastitis strains isolated by this author were similar to those of the S21 series.

Summary.

In a series of 113 cases of mastitis, previously reported and unselected except that they were showing pronounced clinical symptoms, 52 were found to be due to beta-hæmolytic streptococci. In 39 instances these were of the kind commonly met with in mastitis. In the remaining 13 instances the streptococci were distinct from the usual udder streptococci and indistinguishable as a group by bacteriological and serological tests from *Streptococcus pyogenes* of direct human origin. The evidence therefore strongly suggests, though it cannot be held to prove, that in these cases the streptococci had been implanted in the udder by the milker. In seven of the 13 cases the organisms were present in large and predominating numbers, indicating that they were the cause of the mastitis.

Notable features of these strains were their strong hæmolytic properties and their high virulence for mice and rabbits. One strain produced small amounts of soluble toxin. In the more severe cases of streptococcus mastitis, strains with the characters described do not appear to be uncommon.

Enquiry of the veterinary surgeons in attendance elicited the following information. All the cases except four occurred on different farms and in widely separated districts. All the cows had been milked by hand and most of them had recently calved. There was no definite history of illness among the milkers or other persons in contact.

We are particularly indebted to Dr. F. Griffith, Ministry of Health, for supplying a number of streptococcus cultures, and for his opinion regarding some of our strains.

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CONTAGIOUS PNEUMONIA IN SHEEP.

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During the last decade a form of acute pneumonia has caused considerable losses among the sheep flocks in different parts of Iceland, occurring partly sporadically, partly in enzootics, but never uniformly spread over the whole country.

Farming in Iceland is based chiefly upon sheep-breeding, and the income of a farmer depends mainly on the number of lambs he can sell for slaughtering in autumn, this period being the only slaughtering season of the year, when the sheep have returned from the summer pastures in their very best condition. Losses in the sheep-stock, the chief capital of the farming section of the population, are therefore of economical interest, and they were increasing last year, when the disease here described was rapidly spreading in one of the most advanced farming districts of the country.

In spite of numerous outbreaks of this disease no investigations were made into it until the spring of 1929, when two neighbouring farmers in the course of five to six weeks lost 130 sheep, or about 20 per cent. of their stock, during the lambing period. From nine of the dead sheep the lungs and some other organs were sent to our laboratory. The pathological changes, although varying, were evidently of the same nature in all cases, and in seven of the nine cases we isolated a well-defined micro-organism which we supposed to be the cause of the disease. 1 c.c. of an eight hour bouillon culture was injected intratracheally into an old ewe, which for a long time had been kept isolated on a small island. Seven days later the animal was found to be suffering from a heavy dyspnœa. It was killed, and post-mortem examination showed a typical pneumonia, the same kind of micro-organism being cultivated from the lungs. Both lambs of this ewe, injected at the same time in the same manner, showed no signs of illness. These preliminary researches pointed to the isolated microbe as the cause of the disease, but at that time the question could not be definitely settled, as the disease died out with the advent of summer, and a later attempt to infect four sheep with the microbe gave no positive result.

Nothing further was heard of this disease until December of the same year. It then broke out in a few farms not far from the two mentioned above. In the course of the month it spread rapidly, and, as it was causing heavy losses in many farms and the farmers feared for their flocks, I undertook an expedition with the aid of the Government, to make further investigations into the disease. I settled down in the middle of the infected area with an assistant and an equipment for bacteriological work. We stayed there during three months and the following is an account of our findings.

Occurrence of the Disease.—In the district where we were working the same disease had caused great losses on two farms the year before

(See above). On one of these farms ten sheep were lost from the disease this time, but on the other only two were lost out of a similar number (about 300). These two farms seemed to be the centre of the infected area, the disease spreading in all directions from them, hardly any farm in their immediate neighbourhood escaping, but fewer cases occurring with increasing distance.

It is a striking fact that this disease has not been observed in summer, nor in autumn before the sheep are housed. The intimate contact which then occurs evidently plays a large part in the spreading of the disease during outbreaks. It may occur at any time from autumn till spring, but only rarely before the middle of October and after the beginning of June. It has occurred in mountainous districts far from the sea as well as in farms lying close to the coast, where in the winter a considerable part of the sheep's food consists of sea-weed.

Symptoms.—Usually the disease begins suddenly. The first visible symptoms noticed by the shepherd are loss of appetite and a depressed appearance. The loss of appetite is conspicuous at feeding time, when a healthy sheep will rush for its food but a sick one makes no effort to approach the hay offered. The respiration becomes accelerated, the respiratory rate surpassing the normal range of 20-25 and going as high as 50-60 per minute. The body temperature as a rule is elevated, 40-41° C., but this does not occur in every instance. An affected sheep will stand apart from the others, or lie down and remain motionless, avoiding any effort. There may be a slight watery secretion from the eyes, but none usually from the nose, the nostrils only glistening from clear fluid. A seriously affected sheep does not cough. As the disease progresses the sheep is found with the head hanging down, breathing heavily and groaning at intervals. It then falls down and expires. Within twelve hours from the beginning of the disease death may occur, so that sheep which in the evening have eaten greedily in perfect health may be found dead in the morning. The average duration of the disease from the beginning of visible symptoms would be 24-48 hours, but in rare instances a duration of up to 14 days has been observed. Recovery is exceptional. Intestinal symptoms are not prominent, but diarrhœa may sometimes be observed.

Morbid Anatomy.—The chief changes are to be found in the lungs. We have encountered no case of the disease without conspicuous changes in one or both lungs. The parts most frequently affected are the anterior (cephalic) lobe of the right lung and the lower border of the same lobe of the left lung. The obvious predisposition of the right front lobe suggests a respiratory path of infection, this lobe having a bronchial supply of its own, its main bronchus taking origin from the trachea above the bifurcation. The part generally affected in the left lung corresponds to the branching of the first (highest) bronchus which divides from the main stem. Although these parts appear to be specially predisposed to infection,

any part of the lungs may be affected. The changes found are in the form of a more or less extensive consolidation, which may be localised to one continuous area or divided in two, three, or four, or even more places, separated from each other by sound tissue. Sometimes the greater part of a lung may be affected, but as a rule the larger part of the lung tissue is free. Usually not more than one-third of the lung tissue is consolidated, from which the conclusion may be drawn that death is not caused by mere loss of respiratory tissue.

Fig. 1.—Outer aspect of lung from a sheep dead of pneumonia.

The consolidation is of a characteristic nature, so that it can hardly be confounded with any other disease in this country. The pleural

covering of the consolidated area is usually thickened, but in most cases only slightly, so that the colour of the underlying lung tissue is easily seen through. The affected parts are conspicuous through their bluish-red colour, which may be very dark, in places simulating hæmorrhagic infarction. Sometimes, particularly in cases of longer standing, the colour may be lighter, reddish-grey or even of a light grey hue. The consistence is firm, like liver or kidney. On section, the colour may be found varying from reddish-grey to dark-red ; in fresh, acute cases it is more variable, while in older, more chronic, cases the colour is more uniform and greyish. A very characteristic picture is often seen in cases of relatively short duration, when numerous dark-red patches, each surrounded by a slightly elevated greyish wall, are seen on the cut surface. These patches tend to fade in colour in protracted cases, assuming a more uniform greyish colour. A fresh pneumonia may be of variegated appearance from this cause, but, in spite of the difference in colour, no part of the tissue is left unaffected in the consolidated area, the inflammation apparently spreading continuously in all directions. A reddish fluid usually oozes from the cut surface, and on pressure a white or whitish-grey more viscid mucus exudes from the bronchial tubes. The lobular, and sometimes also the lobar, septa may be thick and opalescent from accumulated exudate. The inflamed area is sharply defined from the surrounding tissue, its margins being usually elevated above the sound lung, as a rule by a straight line of bluish colour, and not infrequently forming a clear-cut wall against the sound lung (see Fig. 1), so that tumour growth may be simulated, especially in small consolidated patches, which on all sides are surrounded by normal lung tissue. Subpleural hæmorrhages are frequently seen, not only in the inflamed but also in the parts free from inflammation. In the consolidated areas larger hæmorrhages may be seen, resembling hæmorrhagic infarctions. An advancing inflammation apparently passes over the interlobar septa or other intervening tissue, and in the right front lobe, which is the commonest site, the inflammation often leaves a part of it intact, and on the other side frequently passes directly through the septum on to the next lobe, but it is rarely seen to be confined to a lobe.

The lymphatic glands at the bifurcation of the trachea and in the mediastinum are regularly enlarged, soft and œdematous.

The *trachea* generally contains considerable blood-stained scum, and its mucous membrane is usually heavily injected and velvet-like, but no defects or hæmorrhages are seen.

The *bronchi* are more or less hyperæmic, and contain a white or greyish-white exudate, a little viscid but not very thick.

The *pleura* covering the inflamed tissue is, as already mentioned, usually slightly thickened, but in some cases a thick layer of yellowish coagulated exudate, measuring up to 1 cm. in thickness, is found on the surface of the affected area. Occasionally, especially in protracted cases, an empyema is found.

On the surface of the *heart* small subepicardial petechiæ are

frequently seen, especially on the front side of the left ventricle and along the sulcus coronarius. The heart muscle may be more or less greyish in colour, but the endocardium and valves we never found to be affected. In the *pericardium* we did not encounter inflammatory or other changes, except an occasional slight increase in the pericardial fluid, up to 50 c.c., which was in all cases clear.

Fig. 2.—Divided lung from a sheep dead of pneumonia. Only the central part is free from inflammation.

The *liver* is generally enlarged, the edges being more blunt than normally, but the enlargement is usually not very great. The colour is brown or reddish-brown, the structure on section macroscopically indistinct.

The *gall-bladder* is enlarged and distended, measuring in length from 10-15 cm., the contents rather thin green bile. The enlargement of the gall-bladder is one of the most regular findings at the post-mortem.

The *spleen* is generally not conspicuously enlarged, the capsule showing no signs of any considerable distension. In the reddish-brown pulp the trabeculæ are usually distinctly seen.

The *kidneys* may be slightly enlarged and occasionally a little hyperæmic, but usually no macroscopical changes are seen.

The *adrenals* we have occasionally found hyperæmic.

In the *intestinal tract* we have found no pathological changes except in one case, where a hæmorrhagic œdema of the fourth stomach and hæmorrhagic inflammation of the duodenum and jejunum were found. In this case braxy seemed to have terminated the disease. Anærobic cultivation of the Gram-positive, slender, long rods which were found on the serosa of the inflamed stomach turned out to be *Bacillus welchii*.

In other cases, we found no macroscopical changes in the intestinal tract, but we have not made so many examinations of the intestinal tract as of other organs, the thoracic organs in particular, of which we have carefully examined more than fifty.

The *brain* was examined in three cases only, without any positive results.

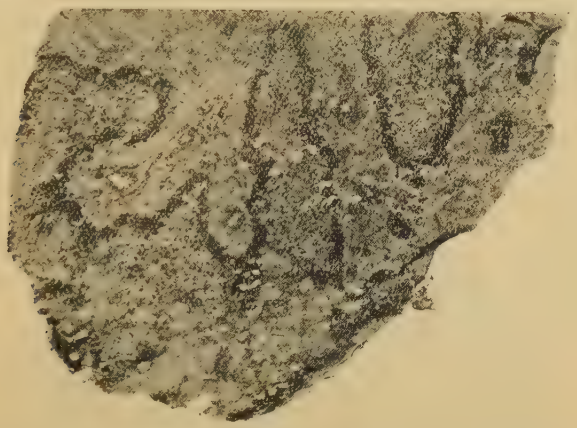

FIG. 3.—Section of inflamed lung (× 5). Note the characteristic figures formed by the leucocytic infiltration. The dark lines correspond to densely packed alveoli.

Microscopy.

Our histological examinations have been restricted to the lungs, where the chief changes are found.

A consolidated area displays a very characteristic picture on microscopical examination. Every alveolus is densely packed with nucleated cells, the interalveolar tissue being œdematous and containing scattered mono- and polynuclear leucocytes. On closer examination the cells contained in the alveoli seem for the greater part to be mononuclear cells, but their nuclei are generally distorted, long and stretched, probably resulting from the pressure exercised one upon the other. Between the cells a small amount of structureless exudate is seen, and in most alveoli some red blood corpuscles, occasionally in considerable numbers. In a fully developed consolidation necrotic parts are seen in the cell-masses of the alveoli, and the necrosis apparently extends as the disease develops, so that extensive necrotic areas are frequently seen. As mentioned above, extravasation

of red blood corpuscles is of common occurrence, sometimes reaching a high degree, resembling hæmorrhagic infarctions. In such parts, we have repeatedly seen necrosis of the affected tissues.

In general we failed to notice a definite relation between the inflammation and the bronchial branches, but occasionally we found a grey hepatisation, several millimetres in thickness, lining a bronchial branch. When dark red hæmorrhagic infiltration is the direct continuance of such hepatisations a characteristic variegated appearance is seen on the cut surface.

In the immediate neighbourhood of a hepatised area the alveoli are usually seen more or less filled with large cells, resembling alveolar endothelial cells, and in most alveoli red blood corpuscles are seen, sometimes in considerable numbers. The inflammatory process seems to begin in the alveoli by an exudation of erythrocytes and mononuclear cells (of the endothelial type) accompanied by some structureless exudate. As the disease advances the alveoli become more and more densely packed with mononuclear cells and some segmented leucocytes, until finally the hepatised tissue may undergo an extensive

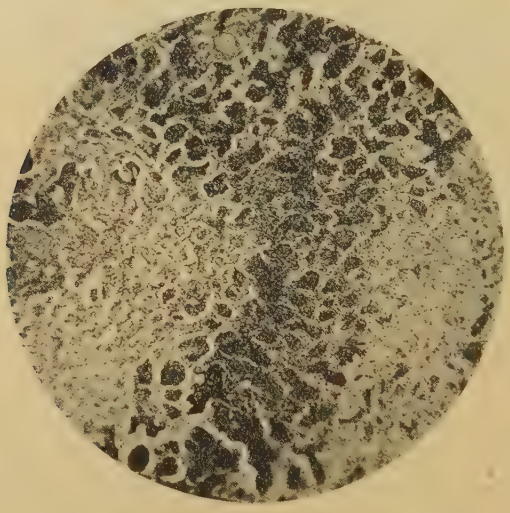

FIG. 4.—Section of inflamed lung (× 40). The central dark part is composed of alveoli occupied by leucocytes. The pale area on the right is in a condition of necrosis, and the one on the left is an early stage of inflammation.

necrosis. Accumulations of polynuclear leucocytes may occur, resembling suppuration, but we have never seen it except in small microscopic areas.

Microscopical examination of stained slides by high power lens system (immersion) reveals a great number of slender rods, 1-3 μ in length. They are seen with some difficulty in densely packed alveoli, more easily in the less infiltrated areas, where they are numerous.

Bacteriology.

Examination of direct smears from the cut surface of a consolidated pneumonia reveals a number of Gram-negative, slender, short rods, most of them 1-3 μ in length, being most numerous in smears from advanced stages of consolidation and comparatively few in those from the first stages of inflammation. These bacilli are as a rule seen in apparently pure culture in the smears, sometimes in enormous numbers. In such smears the bacilli are uniformly stained.

Cultural Characteristics.—The bacilli found in the inflamed parts are easy to cultivate. When a drop of tissue fluid is aspirated by a Pasteur pipette through the heat-sterilised pleura and inoculated in ordinary bouillon and agar, we usually get a pure culture in both media. In six hours the growth is visible in bouillon, and in about twelve hours on agar. The bouillon culture becomes uniformly turbid, but not intensely, the turbidity being rather fine, and without deposit. Pellicle formation we have never noticed. The agar culture shows, after 12 to 16 hours of incubation, greyish lenticular colonies of a blue tint, about 2 mm. in diameter, easily removable, transparent, moist, smooth, and with regular circular outline. The colonies are not viscid or slimy.

When agar-slope cultures are left in the incubator for several days they grow until in five to seven days they become about 5 to 10 mm. in diameter. They are then of a whitish-grey colour, thin, the surface flat and smooth, not wrinkled, the outline circular. A characteristic feature is the form of the margin, which shows a fine radiation, there being a zone of 1 to 1½ mm. in the periphery, where a distinct radiation is seen with the naked eye, this part of the colony being thinner and having a more glistening appearance than the centre.

Under strict anærobic conditions growth is visible on agar plates, but is poor as compared with ærobic growth.

The bacilli are non-motile and, as above stated, Gram-negative. In young bouillon cultures, however, short forms of the bacilli may be found, which resemble elongated cocci and retain the Gram stain. We have repeatedly found a six hours bouillon culture containing for the most part such bacilli, which seem to keep the stain in polar bodies at both ends, but when examined six hours later only Gram-negative rods are seen which contain no polar bodies. We have not been able to find any flagella on the bacilli by the Zettnow method.

We have found no growth in gelatin at room temperature, and at 22° C. no growth occurs, either in gelatine or in bouillon. Gelatin growth in the incubator solidifies when exposed to room temperature.

No indol formation is found in bouillon made of trypsin-digested casein.

Fermentation reactions were made with different carbohydrates and alcohols in a bouillon made of Liebig's meat extract with the addition of ·5 per cent. of the respective sugar and 1 per cent. peptone and bromthymol-blue as indicator, *p*H 7·3-7·5. The fermentations, which are very constant, are seen from the following table, which

is summed up from the registration of 13 different strains observed for a week.

Glucose.	*Lævulose.*	*Mannose.*	*Galactose.*	*Mannite.*	*Lactose.*	*Saccharose.*	*Adonite.*	*Dulcite.*	*Sorbite.*	*Xylose.*	*Arabinose.*	*Rhamnose.*	*Maltose.*	*Salicin.*	*Inosite.*
A	A	O	A	A	O	A	O	O	A	A	O	O	A	O	O

There is no formation of gas in the fermented tubes.

The various carbohydrates are attacked with different rapidity. The first to be fermented is sucrose, which is fermented regularly within 24 hours, then glucose follows, usually within 48 hours. The fermentations of the other carbohydrates usually take place within the first five days, so that the colour of the tubes does not change when a week has passed, but after a week the final result always reads the same. In addition to the 13 strains which were tested with all the above-named carbohydrates, a number of strains in our daily routine work were tested with a smaller number of carbohydrates, using in particular glucose, mannite, lactose, sucrose, sorbite, xylose, and salicin, finding these reactions very useful to identify the bacillus. We found no exceptions in the fermentations from the table given above, the only difference being in respect of the rapidity of the fermentative process, especially as regards mannite and maltose, which varied from one to four days.

Milk is neither coagulated nor acidified by the bacilli. We have never found a strain which fermented lactose, which is of interest in comparison with pasteurella strains described by Magnusson, Eddington and Jones.

On *blood-agar* no hæmolysis was seen, but growth is luxurious in the form of greyish-white colonies.

Influence of Temperature.—The optimal temperature for growth is from 35 to 40° C. Below 25° C. growth is minimal or absent, so that one of our best routine methods of identification is the failing growth in gelatin stabs at room temperature (about 20° C.). A 24-hour bouillon culture was found dead after heating for one minute at 56° C., and 45° C. was tolerated for ten minutes, but not for 20. The resistance to heat is therefore very low. Low temperatures on the other hand are much better tolerated. We have repeatedly isolated the bacilli from lungs frozen up to five days. From a lung kept at 0° C. we did not succeed in cultivating the bacilli after the tenth day, the colonies from the consolidation growing daily scarcer until we got no growth on the eleventh day. Exposure of twelve hour bouillon growth for 15 minutes to a temperature of ± 20° C. was well tolerated.

When left at room temperature a culture very soon dies out. It is usually found dead after two days, but if it be protected from daylight it may live as long as five days. After that time we never found a culture living at room temperature (14 to 20°). In the incubator we have kept a strain now for six months by inoculations every four or five days.

Distribution in the Organs.—We have isolated the bacillus from every case of pneumonia, except in two cases, where the lungs were about a week old and putrid. In fresh, consolidated lungs, one is sure to cultivate the bacilli from the solidified areas, but, as a rule, we have not found them in the apparently normal parts of the lungs. Further, they may, with few exceptions, be cultivated from the enlarged *lymphatic glands* in the mediastinum, as well as from the lymphatic gland, about 10 cm. long, which is found between the lower posterior lobes, and from the smaller ones along the trachea and bronchi. Inoculations from the heart blood are, as a rule, positive from cases dead of the disease. From living cases we have not made many blood cultures, but out of five cases we obtained positive cultures from only two, one heavily attacked ; the other, as well as the negative ones, were from cases which were more lightly attacked. As a rule, the bacilli may be cultivated from the liver also, but not with the same regularity as from the thoracic organs. From spleen, gall-bladder, and kidneys we have also had positive cultures, but not with the same regularity as from the other organs. Only once have we attempted a culture from the brain—with negative result.

Pathogenicity.—We have obtained extensive proof that the described bacillus is the cause of the disease. At the outset of our experiments we secured three sheep, aged respectively two, four, and six years, from a farm far from the infected area, and put them into a house which had been closed and out of use for about a year. Immediately after their arrival the sheep were given 1 c.c. of a 9 hours bouillon culture intratracheally. No change was observed until 21 hours later. The youngest of the ewes then appeared to be very sick, lying still and being unwilling to move, groaning at intervals. No secretion was apparent from nose or mouth, nor was there any coughing. The other two were brisk and apparently healthy, but the respiration was accelerated. The rates of temperature and respiration during the observation were as follows :—

		Jan. 10*th.*		*Jan.* 11*th.*		*Jan.* 12*th.*		*Jan.* 13*th.*	
		9 a.m.	6 p.m.	*Morning.*	*Evening.*	*Morning.*	*Evening.*	*Morning.*	*Evening.*
No. 9	T	40·2	39·4	38·6	Dead				
	R		34	56					
No. 10	T	40·3	40·0	40·5	40·2	40·0	40·4	39·5	39·5
	R		34	46	36	50	40	40	32
No. 11	T	39·4	40·1	40·1	40·0	40·2	40·5	40·3	39·7
	R		40	52	36	40	40	32	32

No. 9 (two years old) was so ill on the morning of January 11th that it could hardly stand, and when raised it staggered and groaned. The temperature was, as seen from the table, not above normal, but the respiration was rapid. At 2 o'clock the same day, *i.e.*, 41 hours after the intratracheal injection, the sheep was moribund. It was then bled. At the post-mortem a typical pneumonia was found in the right lung, which was adherent to the thoracic wall by a thick, fibrin-like yellow layer of exudate. The above described bacilli were obtained in pure culture from lung hepatisation, lymphatic gland from mediastinum, heart blood, liver, kidney, and gall-bladder. All the cultures were examined in bouillon for motility, Gram-stained, and controlled by fermentation tests.

The other two sheep appeared to be in perfect health, moved briskly and showed no loss of appetite. The only change to be found in them was the accelerated respiration. As their condition was unchanged and apparently the disease made no progress, we slaughtered No. 11 when five days had elapsed from the time of the inoculation. At the post-mortem a typical consolidation was found in the inferior border of the front lobe of the left lung, and atalectic, bluish-red patches in the right lung. From the consolidation in the left lung we got pure cultures of the bacilli, also from a hilus-gland, but no growth from heart-blood, spleen, liver, or kidney.

No. 10 was slaughtered on January 15th (six days *post infectionem*). The greater part of the left lobe was found to be densely consolidated by a sharply defined infiltration, which, on section, was light grey-reddish, with dark red patches in it, evidently a typical pneumonia. Culture positive from lung consolidation, heart blood, and gall-bladder, but negative from liver, spleen, kidney, and hilus-gland.

In all three cases the liver was found enlarged and the gall-bladder greatly distended, corresponding exactly to the anatomical changes found in natural infection.

Histological sections from the lung consolidation further confirmed the identity of the disease, displaying the same picture of alveolar infiltration as described above.

Pathogenicity for Other Animals.—Rabbits were injected with 1 c.c. of a fresh 12-hour bouillon culture intravenously, intratracheally, and subcutaneously, without positive result. Cats and dogs proved also to be refractory by the same inoculation methods. Hens were injected intratracheally and subcutaneously without positive result. Two pigeons were not affected by a dose of 1 c.c. subcutaneously.

A pony which was injected with 10 c.c. of a 12-hour bouillon culture intravenously developed a severe chill, shivering and sweating profusely, but after three hours the animal was apparently quite restored.

Two guinea-pigs injected subcutaneously with 1 c.c. broth culture showed no signs of disease after three weeks.

Two mice inoculated with 0·5 c.c. of an 18-hour broth culture succumbed within 20 hours.

Natural Infection.—The described micro-organism is evidently unable to live for any considerable time in free nature, so that infection from grass and hay may be excluded. An investigation into the spreading of the disease revealed, almost without exception, a communication between the infected flocks, so that an outbreak in one place could be traced to a contact with one or more sheep from another infected flock, although not always to sick individual sheep. Of three neighbouring farms two were infected with the disease, but the third farmer took care not to let his sheep mingle with those of his neighbours. One day, at 4 p.m., he found that, during a short absence from his flock, a sick ewe from one of his neighbours had found its way into his flock. He drove it away instantly, and the following morning there were no signs of disease in his flock ; but at 3 o'clock the same afternoon one ewe was severely attacked and died of pneumonia. He then lost one after another from the disease. In other cases healthy sheep from an infected flock have mingled with a sound one, whereupon the disease has broken out, usually after a few days. Outbreaks of the disease have, for instance, frequently occurred on farms, far from infected districts, where apparently healthy sheep from an infected flock had been brought into contact with the healthy one.

These facts pointed plainly to the sheep as spreaders of the disease.

From the nasal secretion of sick sheep we had cultivated the bacillus, so that a respiratory path of infection from sick sheep lay quite near at hand, and may be taken for granted in cases like the above mentioned, where a sick sheep mingles with a sound flock.

Carriers.—But the question remained whether healthy sheep could transmit the disease. In order to make an attempt to settle this question, we made cultures on agar from 20 sheep on a farm where the disease had recently died out. Our search for the bacilli was in vain. Then we took tests from 20 sound sheep on another farm, where the disease had raged the year before and again caused two deaths two months previously. The *B. subtilis* from the hay had been a cause of disturbance in our first attempt, so we took precautions this time, and wiped the nostrils thoroughly with a cotton plug before we inoculated the agar plates. This time we found the typical bacilli in one of the sheep.

Some weeks later we made the same kind of tests on a farm where the sheep were in a bad condition, coughing and ill-looking in spite of plenty of good fodder. Out of 40 tests made in the same manner as before we found two positive.

This showed clearly the existence of healthy carriers, and secondly, that a whole flock may be affected with the disease without any fatal cases, a fact which we had greatly doubted till we obtained this proof.

A question of great importance was whether man could carry the disease. Experience seemed to answer this question in the negative. On several farms where the sheep were kept in two houses the same shepherd foddered in both. But although the disease raged in one of the houses the sheep in the other did not contract the disease until they were let out to pasture and the two groups were mixed. The sheep of the hitherto sound house would then be attacked one after another, the first case occurring from a few days up to fourteen days after the sheep had mixed with each other.

There can be no doubt that healthy carriers play an important rôle in the spreading of the disease. The introduction of one sheep from a previously attacked flock into a sound one has in some cases been followed by an outbreak on the farm in question, so that conveyance by healthy animals is beyond doubt.

Although experience pointed to the contrary, the possibility that man might transmit the disease could not be excluded. But as the distances between the farms in Iceland are generally considerable, and the causative organisms is not tenacious, man is not likely to convey the disease unless the bacilli can live on him and multiply. It was thought that this might take place in his nose, which might possibly be infected if he came into intimate contact with sick sheep or carriers. We made an attempt to solve this question by inoculating agar-plates from the nasal mucosa of six shepherds, all from infected farms, but in no case did we find the bacilli, in spite of repeated tests. Of course, this could not decide the question, but it tended to confirm our opinion that man plays no direct rôle in spreading the disease.

Two sheep-dogs were tested in the same manner, also with negative results.

In some instances outbreaks of the disease had been observed on farms where contact with people or animals was stated not to have taken place for weeks or even months previously to the outbreak. We have not been able to decide this question definitely, but the explanation might be that sheep are infected in summer when flocks from different districts intermingle in the mountainous pastures which are common to all. By this time the resistance to disease is apparently high, for losses have not been observed in summer from that cause, but the rare occurrence in summer may perhaps be explained by the distribution of the sheep, as contagion is evidently far less likely to take place in scattered flocks on pasture than in a crowded house, where an individual animal can hardly escape if there is a source of infection in the flock. Yet the possibility of infection from a carrier in the summer is by no means excluded ; it is even quite probable that it does take place occasionally, and so gives rise to new carriers.

Transmission by insects, especially lice, might come into consideration, and perhaps by birds, but we have made no experiments on the point, nor do we know any facts from practical experience pointing to such an origin of the disease.

Vaccination Experiments.

Some preliminary attempts with carbolised dead vaccine on two infected farms had not given encouraging results, as the disease continued after vaccination just as before. Having repeatedly injected 1 c.c. of a living bouillon culture from 12 to 48 hours old subcutaneously into sheep, which had not been exposed to infection, without any harmful effects except a slight lameness, we wanted to see whether such injections were capable of producing the disease, and, to that purpose, we treated 70 sheep with 1 c.c. each of a twelve hours freshly isolated bouillon culture, using a concentrated beef-peptone-bouillon. The injections were made subcutaneously into the left thigh at 4 p.m. on March 3rd. The following morning all the injected sheep were obviously sick, almost every one being lame, some of them dragging the leg on the side of the injection. Yet there was no inflammation to be seen at the site of injection, or at most only a doubtful one as compared with the other side. The temperature in six of the most sick sheep varied from 39·5 to 40·2, *i.e.*, not above normal. Respiration was considerably accelerated and there was evident loss of appetite in many of them. Two were already dead in the morning after the injection, and two others were bled when moribund three and four days after the injections.

In one of these sheep we found what we had never seen before nor since, namely, multiple small hepatised patches in both lungs, the biggest about the size of a pea, all found immediately under the pleura, probably caused by multiple bacillary emboli. In none of these lungs did we find an advanced consolidation, only atelectatic and hæmorrhagic areas with beginning infiltration. From heart blood and lungs we cultivated the bacilli in all these cases.

The other 66 sheep recovered, most of them showing some lameness after a week, and in many inflammation at the site of injection was considerable at this time, but when two weeks had passed they seemed to have recovered completely.

This method of vaccination was evidently too full of risks to be generally adopted, and even the use of an attenuated living culture would have been difficult to carry out in practice with an organism which soon dies out, especially in a country where rapid distribution is impracticable in winter.

So we turned again to dead vaccines, this time preparing a vaccine from a freshly isolated strain from a lung, growing it in bouillon for twelve hours, and then adding 0·4 per cent. phenol to the culture, keeping it in a dark cold place. After 27 hours the culture was dead.

With similar cultures we vaccinated a number of sheep, some of them twice, and eight days after the latest revaccinations we tested all the vaccinated groups together with a group of twelve treated with living vaccine (1 c.c.) and eleven controls from non-infected farms. All these sheep were injected intratracheally with 1 c.c. of a 12-hour culture from the same flask at 8 a.m. on March 26th. The results are seen from the following table :—

	No. Injected.	*Deaths.*
Living vaccine	12	0
Killed vaccine	30	2
Unvaccinated Controls	11	5

	Injection.	*No. Injected.*	*Deaths.*
Killed vaccine	1 c.c.	16	2
	1 c.c. × 2	6	0
	5 c.c. × 2	4	0
	5 c.c. + 10 c.c.	4	0

This experiment emphasized the usefulness of killed vaccine.

Meanwhile, the disease was continually spreading and causing considerable uneasiness among the farmers, who feared increasing losses as the lambing season was approaching, at which time the ewes are expecially susceptible to infection. We therefore resolved to yield to the wishes of the farmers to supply them with vaccine against the disease, and prepared it on a relatively large scale for all the infected area. We prepared a plan of an organised vaccination, deciding to recommend a general vaccination of sheep in all the infected area, and extending over a good space of " clean " uninfected land. To this purpose we summoned the government representative officials to a meeting where we explained the matter and put forward our plan. The agreement was unanimous. We therefore began at once to produce and distribute the vaccine as above indicated. In all, approximately 50,000 sheep were vaccinated, and about half that number revaccinated after a week's interval. The dose employed was 1 c.c., and the same in case of revaccination.

Course of Disease after Vaccination.—If, as was our original intention, we had left half the stock on each farm unvaccinated the results would have been easier to read. But as the danger was imminent we had to yield to the claims of the people for general vaccination. Only lambs and yearlings were left unvaccinated, as experience had shown them to be but rarely affected by the disease.

Up to the time of vaccination the disease had always been actively at work in one or more places at the same time, having in the course of five months killed about 700 sheep, or averaging 35 per week. The last outbreak occurred on a farm, Brekka, the day before vaccination was carried out, where it killed 40 sheep out of 500 in a comparatively short time. This was the last great outbreak. After vaccination the disease was observed in the following places :—

At *Efranes* 14 died out of 15 attacked by the disease. After vaccination three ewes sickened during the first week, but all recovered.

At *Lundar* one sheep died of the disease twelve days after vaccination. Fourteen had died before.

At *Grund* the disease was observed in one sheep, which died four days after vaccination. The stock was then revaccinated and no further losses were sustained.

At *Mafahlid* the sheep mingled with the infected stock on a neighbouring farm, where 21 sheep had died the previous winter. One sheep from Mafahlid sickened and died a fortnight after vaccination. After revaccination no further losses were noted.

At *Samsstadir* one sheep died after vaccination, having been exposed to cold for two nights.

At *Beigaldi* the disease was observed in twelve sheep a week after the first vaccination. Two were seriously attacked, but only one died.

This makes a total of five* sheep dead after vaccination. When the fact is taken into account that vaccination was finished only three weeks before the beginning of the lambing season, when the susceptibility is at a maximum, and that the losses during the following month were only five as compared with the average 100 during the previous months, the efficacy of the vaccination can hardly be doubted.

Discussion.

The disease in question may be traced back for thirty years in Iceland, and has probably existed there for centuries. The farmers had been so accustomed to losses in their flocks that nobody troubled about it. Braxy was well-known to everybody but, apart from that, most diseases of sheep were thrown into the same category and accounted for by bad forage or starving. Losses during the last weeks of housing (April-May) were usually ascribed to such causes, and, as the pneumonia in question seems to prevail at that time, it might reasonably account for the high sheep-mortality which, although not statistically recorded, has undoubtedly been a matter of fact.

The question arises whether this disease belongs to the group of hæmorrhagic septicæmias, which may occur in outbreaks among sheep. There are many resemblances, both clinical and bacteriological, but many differences according to the literature on hæmorrhagic septicæmia and its ætiological factors in other countries.

Subcutaneous hæmorrhages have not been observed in this sheep-epizootic, nor is there any conspicuous discharge from eyes and nose as described in hæmorrhagic septicæmia. The fact that the oldest animals are picked out by this disease, whereas hæmorrhagic septicæmia chiefly attacks lambs, is a further point of difference. Bacteriologically, too, there are several important differences between the causative bacillus described above and the pasteurella-strains hitherto described.

Jones, finding a new strain of *P. boviseptica* causing pneumonia in cattle, divides the pasteurella causing pneumonia in cattle into three groups, one of which is distinguished from the others since it produces acid in lactose, does not give indol, and is not bile-soluble. The organism here described does not produce indol and is not bile-soluble, but it does not produce acid in lactose and it is not hæmolytic. Its great delicacy, failing to grow at 22° C., its virulence for mice, and its lack of capsules are further points of

* There may have been a few deaths not reported, but at any rate the losses after vaccination were negligible.

difference. To the other two groups it bears still less resemblance. Pneumonia in cattle in England was found by Eddington and Tweed to be caused by a *Pasteurella boviseptica* of Jones Group I. The description given by Eddington of these strains differs from the sheep-strain in question in numerous points. They ferment lactose, arabinose, and inosite ; agar cultures show a yellowish tint which deepens with age, broth cultures show a tendency to form a pellicle and a deposit of granular nature—all properties of which the sheep strain is devoid.

A comparison was then made between a sheep-strain, seven strains of Pasteurella, and one of *B. pseudotuberculosis*, the results of which are seen in the table below.

	Morphology (24 hour broth culture).	*Aspect in 6 days broth.*	*Growth in Gelatin at 22° C.*	*Indol.*	*H_2S*	*Reduction of Nitrates.*	*Growth on MacConkey.*
1. Sheep-strain	Long rods	No deposit	O	O	++	++	+
2. Pasteurella from guinea-pig (*a*)	Long rods	Mucoid deposit	+	+	+	+	O
3. Pasteurella boviseptica (*b*)	Ovoid cocci	Mucoid deposit	+	+	+	O	O
4. Pasteurella lepiseptica (*c*)	Short rods	Deposit	+	+	+	+	O
5. Pasteurella muriseptica	Ovoid cocci	Deposit	+	+	+	+	O
6. Bact. pseudotuberculosis	Rods	Flakes	+	O	±	±	+

FERMENTATION TESTS.

	Glucose.	*Maltose.*	*Lactose.*	*Sucrose.*	*Mannite.*	*Salicin.*	*Inosite.*	*Dulcite.*	*Rhamnose.*	*Arabinose.*	*Xylose.*
1. Sheep-strain	A	A	O	A	A	O	O	O	O	O	A
2. Pasteurella from guinea-pig	A	A	O	A	A	O	O	O	O	O	A
3. Pasteurella boviseptica	A	A	O	A	A	O	O	O	A	A	O
4. Pasteurella leptiseptica	A	A	O	A	A	O	O	O	O	O	A
5. Pasteurella muriseptica	A	A	O	A	A	O	O	O	O	O	A
6. Bact. pseudotuberculosis	A	A	O	O	A	A	O	O	A	A	A

A= Acid. O=No change.

(*a*) From National Collection of Type Cultures, No. 2484.
(*b*) *Ibid.* No. 929.
(*c*) This and the following two strains from London School of Hygiene.

The macroscopical aspect of surface colonies on agar showed no great differences except in the case of *Bact. pseudotuberculosis*, whose colonies were white, dry and wrinkled, in contrast to the grey, transparent and smooth aspect of the others.

Eighteen-hour broth cultures of the same strains (except No. 5)

were injected intraperitoneally into mice, ·5 c.c. of each strain into two mice. Those inoculated with *Bact. pseudotuberculosis* were unaffected, the others succumbed, one after about 36 hours, the others within 20 hours. In none of the dead mice was a pneumonia found. Smears from the heart blood of both mice inoculated with *Past. boviseptica* (N.C.T.C. 929) showed enormous numbers of short ovoid, Gram-negative cocci. In the heart-blood of the other dead mice only a few bacilli were found in the case of strains 2 and 4, but none from No. 1. Smears from the exudate of the peritoneum showed pure cultures of the bacilli and more or less pus. Smears from the pleura and pericardium from strain No. 1 were negative from the one and positive, showing typical pure cultures, from the other.

These comparisons point to a certain relationship between the strains tested, with the exception of the *Bact. pseudotuberculosis*, which, being motile can not be counted to the pasteurella group. Yet, as seen from the table, the sheep-strain displays many points of difference from the ordinary pasteurella (indol negative, growth on MacConkey, no growth in gelatin at 22° C., tendency to die out rapidly at ordinary temperatures.) A further distinguishing feature is its non-virulence for rabbits. It bears certain resemblances to the reindeer-strain isolated by Magnusson, and to the strain from bovine pneumonia described by Eddington. Yet, as shown above, there are several points of difference which distinguish it from the hitherto described strains of pasteurella.

Summary.

An acute, infectious pneumonia in Icelandic sheep is shown to be caused by a strain resembling *Pasteurella*. A description of clinical and anatomical findings is given.

Study of the paths of infection points to carriers as playing an important rôle in the spreading of the disease.

Vaccinations on a large scale were apparently successful.

The biological character of the organism is studied and its relationship to the pasteurella group discussed.

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CHRONIC INFECTION OF THE UDDER OF A GOAT WITH AVIAN TUBERCLE BACILLI.

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In December, 1927, I reported in this Journal the finding of avian tubercle bacilli in the milk of two goats which sometime previously had been inoculated subcutaneously with cultures of avian bacilli.

One goat which had received 20·0 mg. of avian culture was killed 320 days after inoculation. Milk withdrawn by cannula from each half of the udder after the death of the goat was inoculated into guinea-pigs. The guinea-pig inoculated with milk from the right half showed when killed 82 days later a small ulcer and a focus of pus in an inguinal gland ; cultures of avian tubercle bacilli were obtained from the inguinal gland. The guinea-pig inoculated with milk from the left half remained healthy and cultures from its inguinal glands were negative.

The History of the Second Goat.

The second goat, the subject of this report, was inoculated subcutaneously on March 10th, 1926, with 100·0 mg. of an avian strain, and is still alive and well. At the beginning of the experiment the goat was between two and three years old and had had one kid.

The avian bacilli produced at the seat of inoculation on the left side of the neck a very large lesion which sloughed. The resulting excavation gradually healed, and seven months later was represented by a slight thickening of the skin. The left prescapular gland attained the size of a hen's egg, underwent softening, and on February 21st, 1928, as it was threatening to ulcerate the skin, was incised and evacuated. The pus contained moderately numerous tubercle bacilli, which, however, were apparently dead since egg tubes sown with the pus remained sterile. Early in 1929 the right precrural gland was found to be enlarged and fluctuant. On March 14th, 1929, the gland was aspirated, and the pus contained acid-fast bacilli which were apparently dead since they did not yield cultures.

Birth of Kids.

On January 29th, 1927, 10½ months after inoculation, the nanny gave birth to a still-born kid. The organs of the kid were healthy and no tubercle bacilli were found in smear preparations from the placenta and cord near the umbilicus.

On March 15, 1928, the nanny gave birth in the night to two kids, one of which was found dead next morning, having become imprisoned between the wall and a trough ; no sign of tuberculosis was seen in the kid. The second kid was undersized and was reared with difficulty. Tuberculin tests on this kid with avian tuberculin

were negative on two occasions though the nanny reacted vigorously to the same tuberculin. On July 13th, 1928, the kid, then 120 days old, was killed. There was no sign of disease in the organs or glands and cultures from a retropharyngeal and a submaxillary gland remained sterile.

On March 15th, 1930, the nanny again gave birth to two kids, both of which survived. On October 20th, 1930, they, together with the mother, were tested with avian tuberculin. Neither of the kids reacted to the double intradermal test, while the nanny again reacted vigorously to a single injection of the tuberculin.*

One of the kids was killed in November, 1930, and showed on autopsy no sign of tuberculosis. Cultures from a submaxillary and a mesenteric gland remained sterile.

Thus two kids suckled from birth by the nanny showed no sign of infection when killed four and eight months after birth although, as will be seen from the cultivation experiments with the milk, the kids must have ingested with the colostrum milk several thousand avian bacilli.

One may conclude, therefore, that kids are not susceptible to small doses of avian tubercle bacilli taken naturally with the food.†

On the other hand large doses of culture of avian bacilli by the mouth can produce severe local tuberculosis in kids. F. Griffith (1911‡) fed two kids, aged five days, each with 60·0 mg. of avian culture. They were killed in good general health 56 days later and showed extensive ulceration of the Peyer's patches and solitary follicles of the small intestines, enlargement and caseation of the mesenteric and ileo-colic glands, and slight tuberculosis of the tonsils, submaxillary, retro-pharyngeal and portal glands ; there were no disseminated lesions in the organs.

Experiments with the Mammary Secretion.

The goat was " dry " at the time of the original inoculation with the avian culture and the mammary cisterns contained only watery slightly milky fluid. Samples of this fluid withdrawn from each half of the udder at intervals of one, two, three, seven, twenty and twenty-eight days after the inoculation of the goat were tested for avian tubercle bacilli and found to be free of these organisms. The method of testing was by direct cultivation and by making cultures from the inguinal glands of guinea-pigs which had been inoculated subcutaneously with the mammary fluids.

No further tests were made until October 7th, 1926—that is, seven months after the inoculation of the goat. On that date thick milk drawn from the right side of the udder yielded on centrifugation an abundant deposit of slimy pus and showed on microscopical

* I am much indebted to Mr. H. R. Allen, M.R.C.V.S., of the Institute of Animal Pathology, Cambridge, for performing the tuberculin tests on these animals.

† There, is however, a possibility that failure to infect was the result of an immunity transmitted through the mother.

‡ Roy. Comm. Tub. Fin. Rep. App., Vol. iv, p. 190.

examination a moderate number of acid-fast bacilli. The bacilli were obtained in pure culture and proved to be tubercle bacilli of the avian type. From that date to the present day, that is, during a period of more than 4½ years, avian bacilli have maintained themselves in the right half of the udder and have from time to time been recovered in pure culture from the milk.

The cultivation experiments with the secretion from the left half of the udder have been invariably negative. The failure of the avian bacilli to appear in the mammary secretion of this side was at first attributed to atrophy of the mammary gland, but this was not the cause, since after the birth of kids the left half secreted milk in normal quantity.

It will be seen from this table that at the end of each of the " dry " periods avian tubercle bacilli were abundant in the pus-like fluid which was obtainable from the right side of the udder. After the birth of the kids and the elimination of the infective matter which had accumulated in the right udder, avian bacilli were very scanty in the milk during the five or six months when the nanny was suckling her kids. After the weaning of the kids the contents of the right half of the udder, which gradually changed in character from milk to pus-like fluid, became progressively richer in tubercle bacilli and towards the end of gestation again contained extremely numerous avian tubercle bacilli.

The source of the bacilli in the udder is obscure. Manual examination has not revealed any enlargement, induration, or nodular infiltration of the glandular tissue, and therefore one cannot decide whether the bacilli are merely inhabiting the canals of the udder or are being discharged from a small lesion. The paucity of bacilli in the milk during lactation and the absence of signs of tuberculous infiltration of the udder would appear to indicate that there is no lesion and that the bacilli are multiplying on the epithelium of the acini and perhaps also in the cisternal contents. On the other hand it is not unlikely, seeing that the goat reacts well to avian tuberculin, that there is an impalpable lesion in the gland, possibly a superficial ulcer of the acinar or duct epithelium, similar to those which may be found in the intestinal mucous membrane of goats or pigs which have been fed or inoculated with avian tubercle bacilli.

As stated in the previous report, the goat is being kept under observation for the purpose of giving information upon the following questions.

1 *How long will avian bacilli continue to live in the udder of the goat* ?

Precisely when the avian bacilli were first excreted into the milk was not ascertained, no milk tests having been made between the 28th and the 200th day of the experiment. As on the latter date avian bacilli were present in the cisternal fluid in considerable number there is little doubt that they were excreted long anterior to that

The Results of the Cultivation Experiments with the Secretion of the Right Half of the Udder are Summarised in the Following Table.

Date of sowing Cultures.	*Character of the Secretion.*	*Amount of Milk tested.*	*Number of Avian Colonies obtained.*
Six occasions between March 11 and April 8, 1926.	Watery.	Small quantities for direct cultures and 10·0 c.c. for each guinea-pig.	None direct or through the guinea-pig.
October 7, and 8, and December 31, 1926.	Purulent.	A small drop for each egg tube.	Numerous.
	January 29, 1927, *still-born kid.*		
February 21, 1927.	Milk.	One drop.	A dozen.
April 5 and June 21, 1927.	Thick Milk.	„	About two dozen.
September 1, 1927.	„	„	About four dozen.
November 23, 1927 and March 15, 1928.	„	„	Numerous.
	March 15, 1928, *two kids born.*		
March 16, 1928.	Milk.	0·1 c.c. on each of 2 tubes.	Two.
April 16 and June 1, 1928.	„	0·1 c.c. on each of 3 tubes.	None.
June 23, 1928.	„	16·0 c.c. on a G.P.	None from the guinea-pig.
June 25, 1928.	„	40 c.c. on a G.P.	Guinea-pig healthy; avian colonies from the inguinal glands.
August 13, 1928.	„	Two drops.	One.
September 8, 1928.	„	„	Eight.
September 20 and October 16.	„	„	Eighteen to twenty-four.
December 17, 1928.	Thick Milk.	0·1 c.c.	More than 300.
Five occasions from March 11, 1929 to March 10, 1930.	Thick, semi-purulent.	0·1 c.c.	Extremely numerous, uncountable.
	March 15, 1930, *two kids born.*		
June 2, 1930.	Milk.	0·1 c.c.	None.
June 27, 1930.	„	0·1 c.c.	One.
July 14, 1930.	„	0·1 c.c.	Three.
August 14, 1930.	„	0·1 c.c.	Twelve.
September 16, 1930.	„	0·1 c.c.	Twenty-four.
October 16, 1930.	„	0·1 c.c.	Eighty.
November 17, 1930.	„	0·025 c.c.	Innumerable.
December 15, 1930.	„	0·0025 c.c.	Approx. 120.
January 19, 1931.	Thick pus	0·00025 c.c.	Ten to fifteen.
March, 1931.	„	0·0005 c.c.	Twenty-four.

date, probably in the second or the third month. On this assumption, avian tubercle bacilli have been continuously present in the mamma for a period of at least 4¾ years. The goat has now (March, 1931) been " dry " for some months and the fluid in the cistern has again become purulent and rich in tubercle bacilli. Events are thus following the same course as in the previous " dry " periods. It would therefore appear that the infection of the mamma is probably permanent.

2. *If the bacilli persist in the mamma will they eventually destroy the mammary gland tissue*, as was the case in a cow infected with tubercle bacilli of human type (Griffith, 1911)* ?

There is as yet no evidence of a progressive lesion or of destruction of the mammary gland tissue. The milk secreted by the infected half of the mamma after the last birth of kids was abundant and of good quality.

3. *Does the avian tubercle bacillus retain its characteristics during long residence in the tissues of the goat* ?

Cultures of acid-fast bacilli have been cultivated from the milk on numerous occasions—upwards of 30—the last being on March 10th, 1931, that is, five years after the inoculation of the goat. All the strains were identical in cultural characters with the original strain, producing the moist slimy growths of the typical avian bacillus.

The virulence of some of the strains was tested on the fowl and the guinea-pig.

Fowls. Cultures obtained from the milk of the right side of the mamma 2, 3, 4, 4½ and 5 years after the injection of the goat were tested on the fowl. Altogether eight fowls were inoculated, two intravenously and six in the pectoral muscles, and they died in from 24 to 59 days (average 26·5 for intravenous and 40·0 for intramuscular) of miliary tuberculosis of the liver and spleen.

Guinea-pigs. Milk or pus was drawn from the right side of the udder 2¼, 3¼ and 4¾ years after the goat was infected and each sample was injected into a guinea-pig subcutaneously. Two of the guinea-pigs when killed showed no sign of disease. The remaining one, inoculated with the last sample, showed when killed 102 days later a small local abscess and a focus of pus in an inguinal gland on each side ; otherwise the guinea-pig was healthy. Avian colonies were obtained from the inguinal gland pus.

These results show that the avian bacilli have retained their cultural characteristics and specific virulence after a stay of five years in the body of the goat during the greater part of which time they have been multiplying in the udder.

* Roy. Comm. Tub. Fin. Rep. App., Vol. iii, p. 79.

A SPORULATING ANÆROBIC BACILLUS, SIMILAR TO THE CAUSAL ORGANISM OF BLACK DISEASE.

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The bacillus described in this paper has been isolated in four instances from the tissues of sheep which had died suddenly under circumstances where pathogenic anærobic bacteria were suspected as the cause of death, although there was no history of recent parturition or of wounding.

It has been isolated once in over 150 examinations made upon the tissues of sheep from the Romney Marsh, Kent ; but it has been encountered three times in a total of five examinations made upon material received from Wales—twice from the tissues of three sheep from North Wales and once from material from two sheep sent from South Wales.

These figures suggest a peculiar geographical distribution of the bacillus or that the susceptibility of sheep in different localities varies considerably.

In two instances the bacillus was isolated from heart blood and in the remaining two cases from voluntary muscle. In each case the material appeared to be fresh, and microscopical examination showed what might reasonably be considered pure cultures of a Gram-positive bacillus with rounded ends, occurring singly, in pairs, and in short chains or three to four individuals.

Each strain was purified by picking isolated colonies from shake agar cultures and sowing into minced meat medium, from which further shake agar cultures were made and colonies picked, and so on until it was assumed that the cultures were pure.

Morphology. Twenty-four hour cultures in broth containing minced meat gave a large, stout, Gram-positive and irregularly Gram-positive rods with rounded ends up to 12 to 14μ in length. A few bacilli stained irregularly with methylene blue, the organisms having a banded appearance. Occasional bacteria had a subterminal area of constriction. Spores were present in a number of instances. The fully developed spore was of greater breadth than the bacillus. Spindle and citron forms were absent.

Motility.

Motility has not been observed in sealed capillary tubes, or in slide and cover slip preparations sealed round with vasaline.

Cultural characteristics.

The bacillus is anærobic.

Broth containing minced meat.

Growth occurred in this medium with a very moderate production of gas. No colour change was produced in the meat, and the meat was not digested.

Broth.

Growth in medium was not abundant. At first a uniform haze appeared and after twenty-four hours this settled out as a light billowy cloud in the lower portion of the tube.

Milk.

No change was produced in this medium even after prolonged incubation.

Milk containing a portion of brain tissue.

At the end of a week's incubation a soft clot was formed and the expressed whey was clear. Later the clot gradually diminished in size and both clot and whey assumed a buffy tint. At the end of four weeks only a small clot was present in the bottom of the tube, digestion of the clot having occurred to a considerable extent.

Broth containing a portion of brain tissue and a piece of iron wire.

At the end of three days there was slight blackening of the brain and after one week's incubation this was very distinct. In the absence of iron wire no blackening occurred.

Gelatin containing a portion of brain tissue.

The gelatin was slowly liquefied.

Shake liver agar.

Colonies were readily obtained and the majority of these had a compact though irregular centre surrounded by a zone of openly woolly or filamentous growth. Light fluffy or woolly colonies with a compact centre were formed, as were colonies of compact growth surrounded by a number of pin point compact bodies. Simple lenticular colonies, and lenticular colonies with outcrops or tufts of growth form the centres of their biconvex surfaces, were also produced. Fragmentation of the media by gas only occurred when the colonies were very numerous.

Blood agar plates.

Fine delicate irregular colonies were grown ; these were slightly raised towards the centre and had delicate irregular filamentous processes radiating from them. They were surrounded by a zone of hæmolysis.

Serum agar.

Repeated attempts to grow the bacillus on 5, 10, and 30 per cent. serum agar have been made, but growth has only been obtained

in two instances from two of the strains on freshly prepared 10 per cent. serum agar. The colonies, except that they were smaller, resembled those produced by *B.œdematiens*. The serum used in the preparation of the media had been subjected to a temperature of 56° C. for two to three hours. Fresh unheated serum has not been tried. The heating of the serum may account for the absence of growth on serum agar.

No surface growth was formed on Fildes' influenza medium, liver agar, or liver glucose agar, although deep colonies grew readily in the two latter media.

Fermentation reactions.

Glucose, lactose, and maltose were fermented and after growth had ceased these sugars were not demonstrable by the Benedict qualitative method.

There was a doubtful or weak fermentation of glycerine, but the bacilli did not ferment lactose, saccharose, salicin, inulin, or mannite.

Pathogenicity.

0·1 c.c. of a broth culture was pathogenic for guinea-pigs; lesser amounts were not tested. In the inoculated animals an extensive œdema, almost colourless or faintly blood-tinged, was present in the subcutaneous tissues. The lesions were in all respects comparable with those produced by *B. œdematiens*.

Broth cultures of two of the strains were inoculated in amounts of 2 c.c. and 1 c.c. respectively into each of two sheep. In each case death followed in less than 24 hours. The post-mortem examinations were characterised by extensive œdema of the inoculated parts, considerable congestion of the mucosa of the fourth stomach and the small intestine, and in one instance hæmorrhage into the lumen of the bowel. Transudations of fluid were found in the thoracic cavity and the pericardial sac, the amount of fluid in the latter being particularly excessive. Bacteriological examinations failed to reveal the bacteria except in the tissues and fluid around the inoculated parts.

The intravenous inoculation of filtrates of broth cultures into rabbits caused death in two or more hours, depending upon the amount given and its toxicity. At autopsy extensive transudation of pale fluid were found in the thoracic cavity and the pericardial sac, 30 c.c. and 10 c.c. respectively. The lungs were œdematous and frothy. The lesions in these animals were indistinguishable from those produced by *B. œdematiens* toxin.

Immunity.

A formalinised culture of one strain was inoculated intravenously into two rabbits respectively in doses of 1 c.c., 1 c.c., 3 c.c. and 6 c.c. at intervals of two days. Ten days after the final inoculation one rabbit received 1 c.c. of toxic filtrate intravenously and the other 0·5 c.c. of filtrate intramuscularly. Both animals remained well,

but a control rabbit inoculated intravenously with 0·1 c.c. of filtrate died in 10 hours and another receiving 0·5 c.c. intramuscuarly was found dead five hours later. A very complete immunity had therefore been produced in the treated animals.

A few days later serum was collected from the immune rabbits and this serum when mixed *in vitro* with the toxic filtrate of the three heterologous strains and with filtrate of a strain of *B. œdematiens*, obtained from the National Collection of Type Cultures, caused in each instance complete neutralisation of the toxin, a neutralisation not produced by normal rabbit serum. Further, *B. œdematiens* antiserum, kindly given to me by Mr. T. Dalling, of the Wellcome Physiological Research Laboratories, Beckenham, when mixed *in vitro* with the toxic filtrates of the ovine strains of bacteria caused neutralisation of the toxin.

Discussion.

The four strains of the bacillus which have been described show similarities and differences from *B. œdematiens*. In pathogenicity they are alike and the toxin of each is neutralised by the immune serum of the other, but morphologically and culturally distinctions exist which prevent the bacteria being regarded as identical.

The more distinct cultural differences are tabulated below. These may be summarised by saying that the ovine strains studied were less easily cultivated but more proteolytic than typical *B. œdematiens*. Further the ovine strains are distinctly the larger of the two.

	Ovine Strains.	*B. oedamatiens.*
Serum agar slants	Growth not obtained or only obtained with difficulty	Growth easily obtained
Milk	No growth	Growth
Milk plus brain tissue	Casein digested	Casein not digested
Broth containing brain tissue and iron wire	Brain blackened	Brain not blackened

A consideration of the descriptions by Turner & Davesnes (1927) and Turner (1930) of the bacillus of Black Disease reveals much that is common to that organism and the bacilli isolated from sheep in this country.

Thus they both present similar difficulties in cultivation and are alike in size and morphology. Both show a similar range of deep colony types in shake agar and comparable surface colonies on blood agar. They each digest casein and blacken brain in the presence of iron, and ferment glucose, lævulose, and maltose. Their toxins so far have not been distinguished from the toxin of *B. œdematiens*.

Slight differences, however, are to be noted. The strains examined in this laboratory were not observed to be motile, whereas Turner (1930) describes the Black Disease bacillus as sluggishly motile, although motility was not observed by Turner and Davesnes

(1927). Further, the surface colonies of the Black Disease bacillus are not hæmolytic on blood agar, although the toxin is hæmolytic for sheep's erythrocytes *in vitro* (Turner, 1930), but the strains from this country were hæmolytic on blood agar medium.

These differences, however, may be attributable to variation in individual technique and interpretations, and it would appear probable that the strains isolated in this country and the bacillus of Black Disease are like variants of *B. œdematiens*.

At present Black Disease is only reported from Australia and New Zealand, and possibly Tasmania, and it is believed that the presence of immature liver fluke in the hepatic tissues function as activators for the spores of the bacillus lying latent in these tissues and that after activation localised areas of bacterial activity give rise to necrotic hepatitis, toxæmia, and death.

In view of the prevalence of liver fluke disease it may be reasonable to suppose that outside certain districts in the aforementioned countries the spores of the causal micro-organism of Black Disease are not present in a quiescent state in the livers of sheep, or that Black Disease, if it occurs in other parts of the world, passes without recognition.

Summary.

From material received from a total of five sheep which came from Wales a bacillus similar to the *B. œdematiens* type of micro-organism which is held responsible for Black Disease has been isolated in three instances. The same bacillus has been isolated in one instance only from material from over 150 sheep from the Romney Marsh, Kent.

These findings suggest a peculiar local distribution of the micro-organisms. If such be the case, does Black Disease occur in this country in districts where the liver fluke and the *B. œdematiens* type of bacillus occur, or does this micro-organism cause disease in sheep independently of any liver fluke disease ?

References.

Turner, A. W., and Davesnes, J. 1927. Ann. Inst. Pasteur, 41, 1078.
Turner, A. W. 1930. Bul. No. 46. Council for Scientific & Industrial Research, Australia.

Note.—Since the above paper was written an article by H. Miessner, A. Meyn, and G. Shoop, Centralt. f. Bakt., 1931, 120, p. 257, has been brought to my notice. In this article bacilli of the *B. œdematiens* type, and probably identical with the bacillus of Black Disease, are considered to be the cause of Bradsot in certain districts in Germany. In cases examined before putrefactive changes were advanced necrotic areas were found in the liver. These were regarded as similar to the liver lesions in Black Disease. However, no co-relation between liver fluke infestation and Bradsot was established, although some of the animals harboured the parasites.

REVIEWS.

Die Rhacitis in ihren ätiologischen, biochemischen, pathogenetischen, pathologisch-anatomischen und klinischen Beziehungen. Eine experimentelle und vergleichende Studie. Von Dr. Josef Marek, o.ö Professor der Speciellen Pathologie und Therapie und o.ö Dr. Oskar Wellmann, Professor der Tierzuchtlehre, an der Veterinär-Hochschule zu Budapest. **Pathologischer Teil,** von Dr. Josef Marek. Jena : Gustav Fischer, 1931. Price. 24 marks.

As the title indicates, the present volume, by Professor Marek, sets forth the pathological part of the combined researches of the two authors, but it incorporates the essential parts of the chemical researches by Professor Wellmann, which will form a separate volume.

The results relate to 33 cases of spontaneous rachitis in children, calves, dogs, pigs, lambs, and kids, and experimentally produced rachitis in 21 puppies and 41 pigs.

The work has 344 pages, and is divided into six sections, which deal respectively with : Spontaneous rachitis, experimentally produced rachitis, rachitis in the domesticated animals compared with that in man, the pathogenesis and histogenesis of rachitis, the ætiology of rachitis in the light of recent research, and the cure and prevention of rachitis. There are 171 figures in the text, including photographs and X-ray pictures of the diseased bones and micro-photographs of the lesions. There is at the end a useful Index, and a list of references (322) to the most important books and articles on rachitis.

The experiments appear to have been planned and executed in a way that might serve as a model for such researches and the manner in which the evidence derived from them is presented is admirable. The author's principal conclusion is that in the domesticated animals as a rule the determining cause (äuslösende Schädlichkeit) of rachitis is a deficiency of calcium or of phosphoric acid in the bodies of animals fed on unsuitable diet. This applies to sporadic cases as well as to outbreaks. A lack of Vitamin D is seldom a cause in these animals, and even then is usually combined with the more important mineral deficiency. This view of the ætiology of the disease in animals cannot be ignored on the assumption that rachitis in the lower animals is a process different from the one so-named in man, for Professor Marek gives good reasons for maintaining that the alterations are essentially of the same nature. He suggests that further investigations may show that in the human subject also a deficiency of calcium and phosphoric acid may play a larger part in the genesis of rachitis than is generally attributed to it at the present time.

A point deserving emphasis is that by means of X-ray examination of the living animals it was possible to show that a diet effectively rachitogenous could be made curative without any alteration except a correction of the defect in its mineral constituents. The basal diet used in the experiments on pigs was composed of the ordinary food of these animals and was practically free from Vitamin D.

The work is not the last word on the subject, but it marks a great advance in knowledge regarding the ætiology of rickets in the domesticated animals, and therefore of the means by which the disease can be prevented and cured.

Lehrbuch der speciellen pathologischen Anatomie der Haustiere. Von Dr. med. vet. Karl Nieberle und Dr. med. vet. Paul Cohrs. Jena : Gustav Fischer, 1931. Price, 48 marks.

This imposing book extends to 796 royal 8vo pages, and has 556 illustrations. The work has been almost equally divided between the two authors, both of whom hold chairs in the Institute of Veterinary Pathology in the University of Leipzig and are well known authorities on the subjects on which they write. The systems of the body are dealt with separately, and the morbid conditions occurring in each are very fully described in regard to both gross and microscopic characters. The work has been designed mainly for students and practitioners, and is, to a large extent, based on the experience of the authors themselves. On that account, they have not burdened the text with many references.

It goes without saying that a critic might here and there find himself in disagreement with the authors, but as an exposition of the present position of veterinary pathology the work is on a high level, and reflects the wide knowledge and experience of the authors. One could hardly recommend it as well adapted to the needs of ordinary students, but it certainly should have a place in every veterinary or medical library, and no teacher of pathology can afford to be without it. Both authors have a style that is uninvolved and makes easy reading, and the publishers' share of the work deserves praise.

The Veterinary Bulletin, April, 1931. Published by the Imperial Bureau of Animal Health, Weybridge, Surrey.

This is the opening number of a new publication which succeeds the Tropical Veterinary Bulletin, the last number of which appeared in December, 1930. It gives effect to a recommendation made by the Imperial Agricultural Research Conference in 1927 that a more comprehensive journal should be issued by the Imperial Bureau of Animal Health. The new Bulletin will deal with all aspects of animal health in so far as original research and administrative control are concerned with them, but not with clinical material from the point of view of the practitioner. Diseases will come under review irrespective of the climate under which they occur, and each volume after the first will run to about 600 pages. For the current year the journal will be published quarterly, but afterwards in monthly parts, price £1 per volume.

The initial part contains abstracts of over 170 articles covering a very wide range of diseases in all parts of the world, and it reflects credit on the Editor and other members of the staff of the Imperial Bureau of Animal Health. The journal deserves the best wishes of all who are interested in the advancement and diffusion of knowledge regarding animal diseases at home and abroad.

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